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ON THE MORPHOLOGY AND ONTOGENY OF THE FOLIAR SCLEREIDS OF *CODIAEUM VARIEGATUM* BLUME

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ABSTRACT

The occurrence and distribution of sclereids in the leaves and petals of *Codiaeum variegatum* var. *molluccanum*, horticulturally known as var. *neo-guineense* was studied.

The petals have sclereids in the form of thick walled hairs which are transformed epidermal cells. The leaves and prophylls have a network of very long and branched sclereids with thick lamellated walls. An ontogenetic study shows that these are transformed laticiferous vessels. Such sclereids are also present in the petals but their walls are not so much thickened. These branched sclereids could, on the basis of their shape, be placed under the type "*astro-sclereids*" or "*polymorphic sclereids*" of various authors.

So far as the authors are aware this is the first report of the occurrence of sclereids in *Codiaeum variegatum* var. *molluccanum* and also of the conversion of laticiferous vessels into sclereids in this genus.

INTRODUCTION

The study of foliar sclereids in Indian angiosperms has not only been a recent aspect of botanical study but is also confined mostly to the work of Rao (1951, 1958), Subramanyan and Rao (1949), Krishnaswamy (1942), Rao and Kulkarni (1952) and Rao and Kelkar (1951). The subject however is interesting not only from the histological and anatomical point of view but is also of taxonomic importance, particularly in the study of sterile material of spermatophytes. This study is also likely to be of very great value to the palaeobotanist who has to deal with fragments of plant material. In view of the importance of this subject a series of investigations on the sclereids of some select Indian types have been undertaken. And the present paper is the first of the series and embodies our observations on *Codiaeum variegatum* Blume var. *molluccanum* Muell. a member of the Euphorbiaceae and horticulturally known as a variety *neo-guineense*.

MATERIALS AND METHODS

Leaves and petals were studied and collected from the plants growing in the departmental garden.

Hand sections as well as microtome preparations, macerations and cleared whole mounts of leaves of different ages were examined.

Leaves were macerated by keeping them in concentrated nitric acid for 3 or 4 days and then were teased out.

For making cleared whole mounts leaves of various ages were dehydrated in normal series of alcohol, cleared in xylol and mounted in Canada balsam. However excellent clearing of the leaves was obtained when they were kept for some time in concentrated nitric acid prior to dehydration. The cleared whole mounts were studied unstained.

The plant under investigation has numerous laticiferous vessels. This was confirmed by staining the sections in dilute aqueous iodine solution followed by

aqueous cosine or erythrosin. Then, the latex tubes stain rose-pink and the starch grains purple. This is a method recommended by Gatenby and Painter (1937, p. 647). The sections were directly mounted in 2 per cent aqueous acetic acid or dehydrated, cleared and mounted in Canada balsam.

Safranin, Gentian Violet and Haematoxylin were the stains used. A clove-oil stain was also used often for counterstaining. Temporary mounts were stained with Cotton Blue.

MORPHOLOGY AND DISTRIBUTION OF THE SCLEREIDS

Sclereids in petals : These may occur in the form of unbranched, thickwalled irregularly arranged hairs distributed on the inner surface of petals. Each such sclereid (Fig. 1) consists of two portions—a rounded base embedded in the petal tissue and a cylindrical body straight or variously curved and tapering to a point. The lumen compared to the size of these sclereids is small. The thick wall of the sclereid is homogenous, and unlamellated.

Some long and branched sclereids were also found in, the petals. These, like the ones in the leaves (discussed below), are latex carriers at first but later the contents gradually disappear and they become slightly thick walled.

Sclereids in leaves and prophylls : These are numerous and branched in the leaves and prophylls as well. Fig. 2 shows a diagrammatic representation of the distribution of sclereids in a leaf as seen in a cleared leaf mount. The sclereids form a continuous submarginal series as seen in Fig. 2 and Fig. 1, Plate I. Further, they are distributed in a direction at right angles to the midrib. A few of these are also irregularly oriented. Fig. 3 is an enlarged sketch of a part of the lamina showing a bifurcating vein accompanied by mostly parallel but also irregularly oriented sclereids disposed either solitarily or in bundles. The sclereids can be seen to be very long sinuous often completely bent and the walls are heavily lamellated. These lamellations numbering three to four, often more, are seen clearly in transverse sections of the marginal sclereids seen in a cross-section of a leaf (Fig. 4). The sclereids as seen in surface studies are very long—nearly one sixth of the entire length of a lamina. Sclereids in older leaves often show small protuberances to various extent looking almost like spines (Fig. 5), or stunted branches (Fig. 6, 7, and 8). Such branched sclereids are found in the spongy mesophyll of the leaf (Fig. 9 and Fig. 2, Plate I), their branches often running into the palisade tissue.

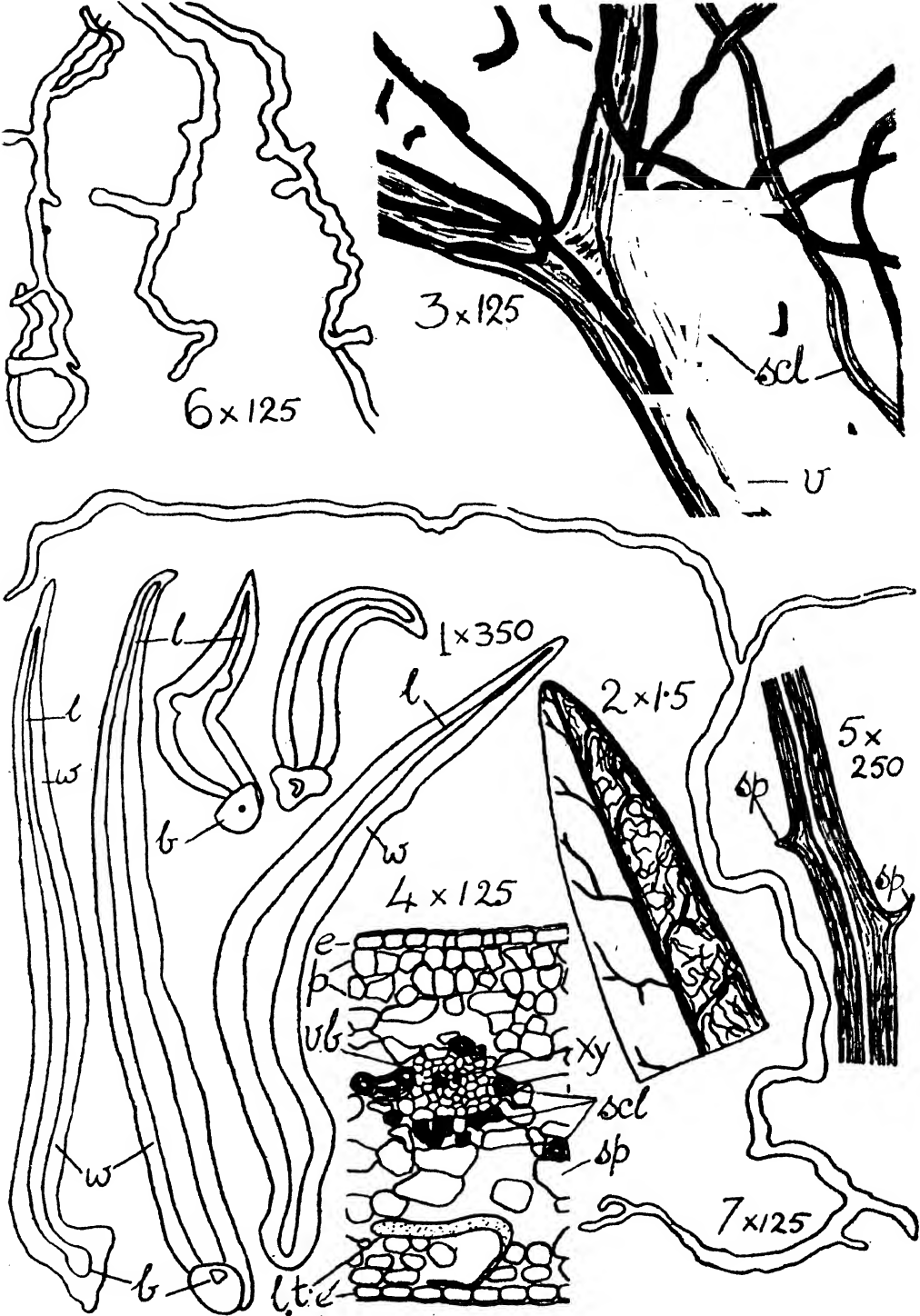
Sclereids in petioles and in the midrib are often shorter in length, unbranched and are mostly distributed in the cortex (Fig. 10). Even here they may be solitary or in bundles. They are however shorter than those in the leaves (Fig. 11).

ONTOGENY OF THE SCLEREIDS

So far as observed, the sclereids, discussed above, leaving aside the epidermal hairs, are not cut off from any procambial strand, but are converted laticiferous

EXPLANATION OF TEXT-FIG. 1

1. Sclereids on petals, b-bulbous base, l-lumen, w-thick wall.
2. Distribution of sclereids as seen in a cleared leaf mount.
3. A part of the above drawn on a large scale to show the branched vein-v and sclereids-scl., some running with the vein and others irregularly disposed.
4. Transverse section of leaf showing sclereids with lamellated walls e-upper epidermis, 6-lower epidermis, l.t.-laticiferous tube, p-palisade, scl-sclereids, sp-spongy parenchyma, vb-vascular bundle, xy-xylem..
5. Part of an old sclereid showing spine-like out growths, sp-spine.
6. Parts of old sclereids showing stunted irregular branches.
7. Another old and very long branched sclereid.



TEXT-FIG. 1.

vessels. In very young stages these sclereids are thin walled, large lumened and have inclusions which answer to the latex test as stated above. Some of the branched laticiferous vessels in their earlier stages (Figs. 12 and 13) have inclusions, are slightly brittle and even break apart into pieces. Later, the latex is absorbed and the walls become stratified (Fig. 14) by a deposition of several lamellae of a substance whose nature could not be satisfactorily ascertained. Several such imperfect sclereids, parts of which are thin-walled and laticiferous and parts of which are devoid of latex prior to sclerization, were observed generally in the spongy mesophyll of the leaves. But the laticiferous vessels that are generally distributed in the palisade remain laticiferous except very rarely when they too are converted into sclereids (See Fig. 9). It thus appears from ontogenetic studies that the sclereids are essentially converted laticiferous vessels. Similar phenomenon has been observed by Rao (1951) in *Hoya pauciflora*, a member of the Asclepiadaceae. But so far as we are aware this has not been reported in the Euphorbiaceae.

These branched sclereids could be placed under *Astero sclereids* (Tschirch, 1885 p. 301-2; Foster, 1942, p. 68; Eames & MacDaniels, 1947, p. 89; and Esau, 1953, p. 213). They also agree in general form with the polymorphic type of sclereids, regarded as modified spongy cells and placed by Rao (1951, page 32) under Group III (Type IV).

The sclereised epidermal hairs on the other hand arise as small papillae and continue to grow as short thin walled protuberances upto a certain length (Fig. 15). They continue to grow upto a particular stage (Fig. 16). Rarely a cross-wall may also appear and the papilla may become a two celled-filament (Fig. 17). At this stage the cells are densely protoplasmic with wide lumen and thin walls. As soon as their growth stops the walls become thickened homogeneously although they do not show any stratification of the wall layer as in the foliar sclereids.

These transformed epidermal hairs come under Group I of Rao (1951).

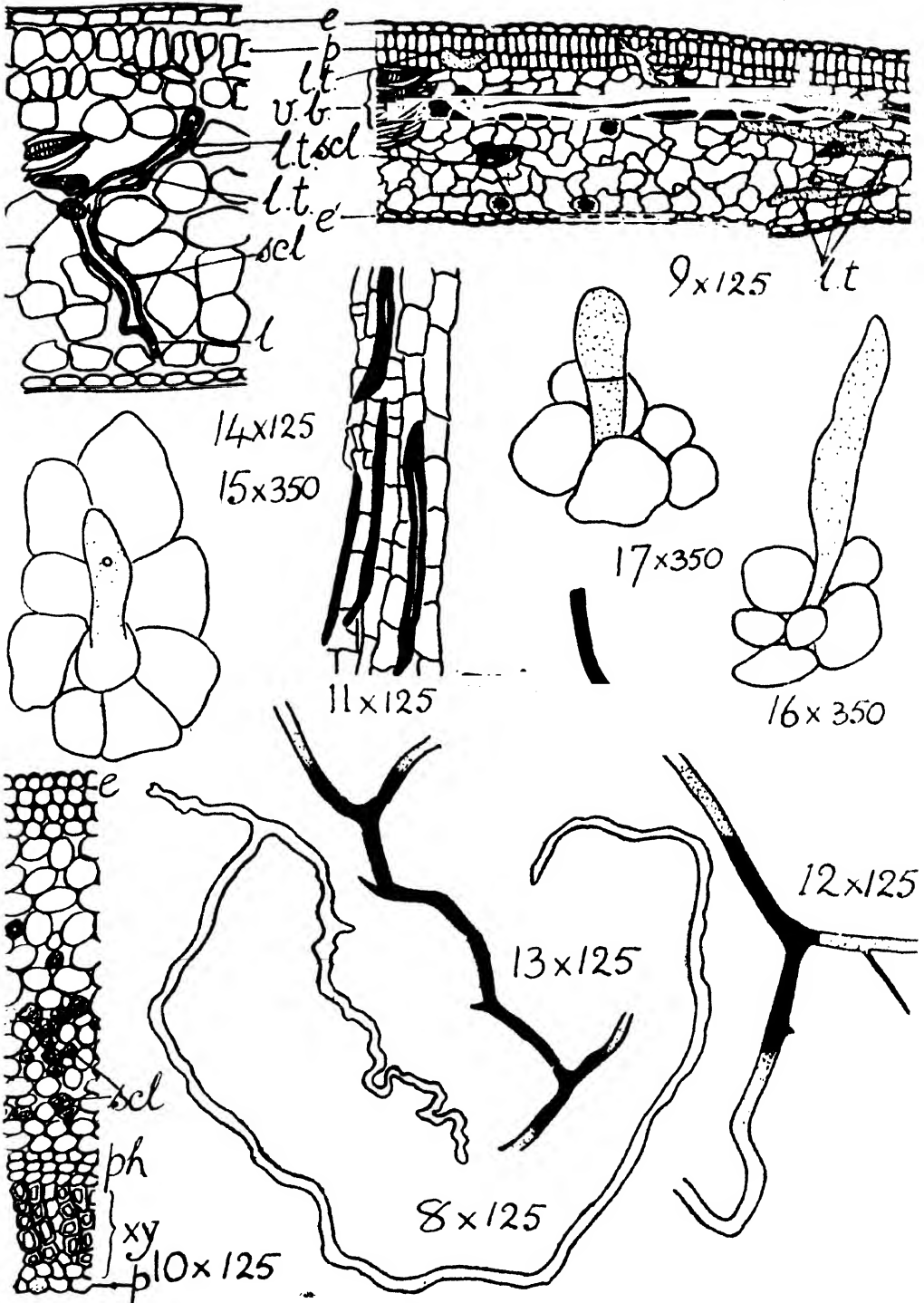
ACKNOWLEDGEMENT

The authors are grateful to Mr. Lancaster of the National Botanical Gardens, Lucknow, who identified the variety *neo-guineense* for them. They are greatly indebted to Dr. T. A. Rao who very kindly and readily helped them with the literature. They also thank Mr. S. K. Nath, who took the photographs 1 and 2.

EXPLANATION OF TEXT-FIG. 2

8. An old and very long branched sclereid.
9. Transverse section of leaf showing sclereids and branched laticiferous tubes in the mesophyll. A small sclereid is seen in the palisade. e-epidermis, é-lower epidermis, p-palisade, lt.-latex tube, scl-scleroid and v.b.-vascular bundle.
10. Part of a section of petiole showing sclereids in the ground tissue cut transversely. Note that sclereids occur solitarily or in bundles. e-epidermis, scl-sclereid, ph-phloem, xy-xylem, p-pith.
11. Part of a longitudinal section of petiole showing short sclereids.
12. and 13. Branched laticiferous tubes with partly preserved inclusions or latex shown black. The laticiferous tube shown in Fig. 13 is breaking up into pieces.
14. Transverse section of leaf showing a sclereid formed from a laticiferous tube. The latex contents can be seen in the lumen scl-sclereid, latex contents.
- 15, 16. and 17. Hairs (sclereids) on petals in different stages of development.

Pl 2



TEXT-FIG. 2.

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EXPLANATION OF PHOTOGRAPHS IN THE PLATE

1. A small portion of a leaf cleared to show the disposition of the sclereids. Note that the sclereids run parallel to the midrib and become crowded at the periphery. $\times 135$.
2. Part of the transverse section seen in fig. 9 photographed to show the thick lamellated walls of a sclereid (scl) in the spongy mesophyll. A latex tube (lt) seen in the palisade in fig. can also be made out in the right side of the photograph. $\times 540$.

NOTES ON SOME OEDOGONIALES FROM KERALA STATE, INDIA

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ABSTRACT

Three species of the genus *Bulbochaete* and fifteen of *Oedogonium* are described from Kerala State, of which one species of *Bulbochaete* and one variety and one form of *Oedogonium* are new. An emended description of *Oedogonium perspicuum* Hirn is given and *Oe. keralense* is reduced to a variety of *Oe. perspicuum* Hirn. The world distribution of the forms is also given.

INTRODUCTION

The collections on which this study is based were made by Dr. M. S. Randhawa from Kerala State during January 1959. To Dr. Randhawa we extend our grateful thanks for the opportunity of working on this material. The most striking feature of these collections is the preponderance of Oedogoniales. However, it is difficult to form any definite idea of the algal flora of the region from few samples and it is earnestly hoped to get more material at some future date. The desirability of survey of this region, which remains largely unexplored, is strongly indicated.

In all 18 forms are described in this paper including 3 of *Bulbochaete* and 15 of *Oedogonium* of which 1 species of *Bulbochaete* and 1 variety and 1 form of *Oedogonium* are new. *Oedogonium keralense* described by Erady and Rajappan (1958) from this region has been reduced to the rank of a variety of *Oe. perspicuum* Hirn and the description of the type is emended. Critical notes and the general distribution of the forms are also given for the forms reported here.

GENUS *Bulbochaete* AGARDH

1. *Bulbochaete intermedia* De Bary. Hirn. 1900, p. 326, t. LII, figs. 333-335; Tiffany, 1930, p. 35, t. I, fig. 5; 1937 II, p. 8, t. II, fig. 14; Gemeinhardt, 1939, p. 379, fig. 466.

Dioecious, nannandrous, gynandrosporus: Oogonia subdepressed globose, patent, below androsporangia; division of the suffultory cell median; Oospore same form as the oogonium, outer spore wall scrobiculate; androsporangia 1-3, epigynous or scattered; nannandria on oogonia, stipe slightly curved; antheridium interior (figs. 6-7).

Vegetative cells $12-16 \times 32-64\mu$; Oogonia $40-44 \times 32-36\mu$; Oospores $38-42 \times 28-32\mu$; androsporangia $11.4-13.3 \times 7.6-11.4\mu$; dwarf male $8-10 \times 12-16\mu$.

The present form differs from the type and var. *depressa* in its slightly smaller dimensions.

Habitat: Epiphytic on aquatic plants along with *B. varians* var. *subsimplex*. Krishnapuram, Kerala, January 13, 1959.

Distribution: Africa, America, Australia, China, England and Europe.

2. *Bulbochaete varians* Wittrock var. *subsimplex* (Witt.) Hirn. Hirn, 1900, p. 357, t. LX, fig. 334; Tiffany, 1930, p. 45, t. VI, fig. 49; 1937 II, p. 13, t. V, fig. 48; Gemeinhardt, 1939, p. 406, fig. 502.

Diocious, nannandrous, gynandrosporus; Oogonia ovoid to ellipsoid, patent or erect, below terminal setae or androsporangial cells or very rarely below vegetative cells; division of the suffultory cell supreme; Oospore same form as the oogonium, wall of the oospore longitudinally ribbed; androsporangia epigynous or scattered, 1-2; dwarf males on or near the oogonia; antheridium exterior, 1-3 (fig. 5).

Vegetative cells $15.2-19 \times 19-22.8\mu$; Oogonia $26.8-30.4 \times 38-45.6\mu$; Oospores $22.8-26.6 \times 35-43.7\mu$; androsporangia $11.4-13.3 \times 7.6-11.4\mu$; dwarf male stipe $11.4-15.2 \times 22.8-38\mu$; antheridia $7.6-11.4 \times 3.8-7.6\mu$.

Habitat : Epiphytic on aquatic plants along with *B. intermedia*. Krishnapuram, Kerala, January 13, 1959.

Distribution : Australia, Brazil, British Columbia, China, India (Calcutta and Bombay), Europe and United States of America.

3. *Bulbochaete keralense* sp. nov.

Diocious, nannandrous, idiandrosporus; vegetative cells cylindrical, longer than broad; Oogonia depressed-globose, erect, below a terminal seta very rarely below vegetative cells; division of the suffultory cell basal; Oospore of the same form as the oogonium, completely filling it, oospore wall scrobiculate; androsporangia 1-4, occurring on lateral branches, either terminated by a seta or intercalary; dwarf male situated on or near oogonia, on the terminal cells or very rarely on the intercalary vegetative cells; dwarf male stipe slightly curved, occurring in clusters; antheridia exterior, 1-2 (figs. 1-4).

Vegetative cells $11.4-15.2 \times 22.8-45.6\mu$; Oogonia $34.2-38 \times 30.4-34.2\mu$; Oospores $32.3-36.1 \times 28.5-32.3\mu$; androsporangia $11.4 \times 11.4\mu$; dwarf male stipe $11.4-13.3 \times 22.8\mu$; antheridia $7.6 \times 3.8-7.6\mu$.

Diocia, nanandra, idiandrospora; cellulae vegetativae cylindricae, longiores quam latiores; oogonia depresso-globosa, erecta, infra setam terminalem, raro infra cellulas vegetativas; divisio cellula suffulcientis basalis; oospora eiusdem formae ac oogonium, penitus implens oogonium, parietibus scrobiculatis; androsporangia 1-4, ramis lateralibus insidentia, tum desinentia in setam tum intercalaria; mares nani prope oogonia vel iis insidentes, super cellulas terminales vel rarissime super cellulas intercalares; eorum vero stipites tenuiter curvati, fasciculati; antheridia exteriora, 1-2. Cellulae vegetativae $11.4-15.2 \times 22.8-45.6\mu$; oogonia $34.2-38 \times 30.4-34.2\mu$; oospora $32.3-36.1 \times 28.5-32.3\mu$; androsporangia $11.4 \times 11.4\mu$; stipes maris nani $11.4-13.3 \times 22.8\mu$; antheridia $7.6 \times 3.8-7.6\mu$.

This form comes very close to *B. scrobiculata* Tiffany in its scrobiculate oospore wall but differs from the same essentially in the idiandrosporus nature of the plants. From the other idiandrosporus species of this genus having scrobiculate oospore membrane, this form can be easily distinguished by the basal division of the suffultory cell.

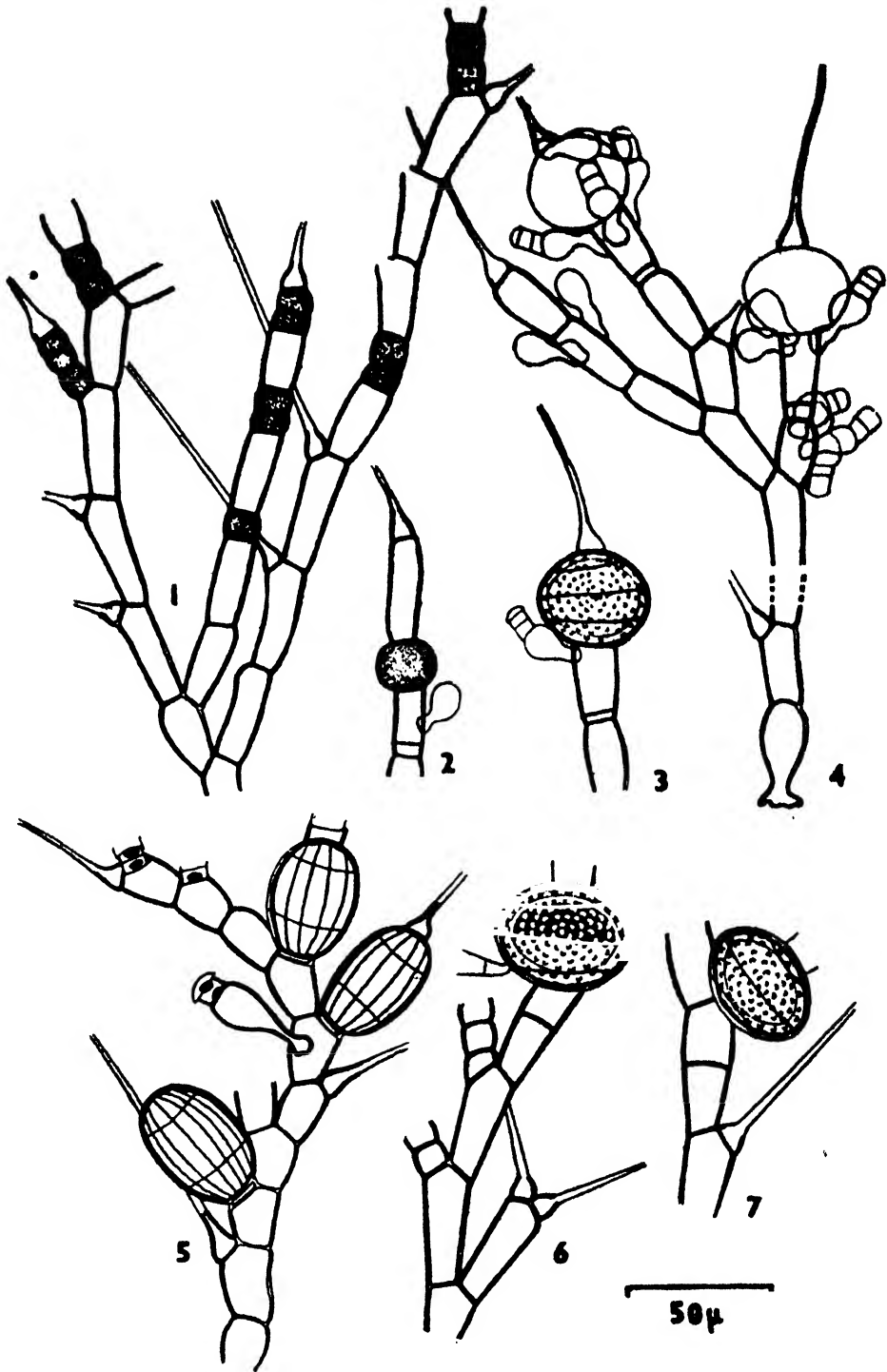
Habitat : Epiphytic on aquatic plants. Kottarakara, Kerala, January 15, 1959.

Typus : Randhawa collection BKI-159, Indian Agricultural Research Institute, New Delhi, India.

GENUS *Oedogonium* LINK

4. *Oedogonium perspicuum* Hirn char. emend. Venkatarāman & Natarajan (Hirn, 1900, p. 273, t. XLVI, fig. 293).

Plants at first attached by means of lobed elongated obovate hapteral cells. Diocious, nannandrous, idiandrosporus; Oogonia solitary or 2-9 seriate, terminal



Figs. 1-7. *Bulbochaete keralense* sp. nov. 1, part of the androsporangial plant; 2, an immature oogonium below a vegetative cell; 3, oogonium with a mature oospore showing the scrobiculate spore wall; 4, female plant with basal cell, oogonia and dwarf males; Fig. 5, *B. varians* var. *subsimplex* (Wittrock) Hirn.; Figs. 6-7, *B. intermedia* De Bary.

or intercalary, depressed or sub-depressed globose or sub-depressed-pyriform-globose, operculate, division median, wide; Oospore globose or rarely subglobose, not filling the oogonium or filling the oogonium to a fair extent, but not fully, spore wall smooth; suffultory cell same as the vegetative cells; androsporangia 2 to many seriate; nannandria unicellular, on oogonium, rarely on suffultory cells or sometimes even on vegetative cells; terminal cell broadly rounded (figs 8-11).

Vegetative cells (26) $34.8-45.6 (40.4) \times 11.4-14.6 (17.1) \mu$; suffultory cells $34.2-49.4 \times 57.4-114 (152) \mu$; Oogonia $83.6-91.6 \times 60.8-79.8 \mu$; Oospores (57) $60.8-76 \times (57) 60.8-76 \mu$; androsporangia (19) $26.6-38 \times (7.6) 11.4-19 \mu$; nannandria $15.219 (22.8) \times 19-26.6 \mu$.

Habitat : Initially attached to the aquatic plants, later becoming free-floating in freshwater puddles, Kottarakara, Kerala, January 15, 1959.

Typus : Randhawa collection OKI-162, Indian Agricultural Research Institute, New Delhi, India.

Plantae primo fixae per cellulas hapteras lobatas elongatas obovatas, dioicae, nanandrae, idiandrospora; oogonia solitaria vel 2-9 seriate, terminalia vel intercalaria, depresso-globosa vel subdepresso-globosa, vel subdepresso-pyriformiglobosa, operculata, divisione media, lata; oospora globosa raro subglobose, oogonium non implens vel idem implens plus minusve sed non penitus, parietibus levibus-cellulae suffulcientes eadem ac cellulae vegetativae; androsporangia bis ad pluries seriate; nannandria unicellularia, oogonio raro cellulis suffulcientibus vel nunquam cellulis vegetativis insidentia; cellula terminalis late rotundata.

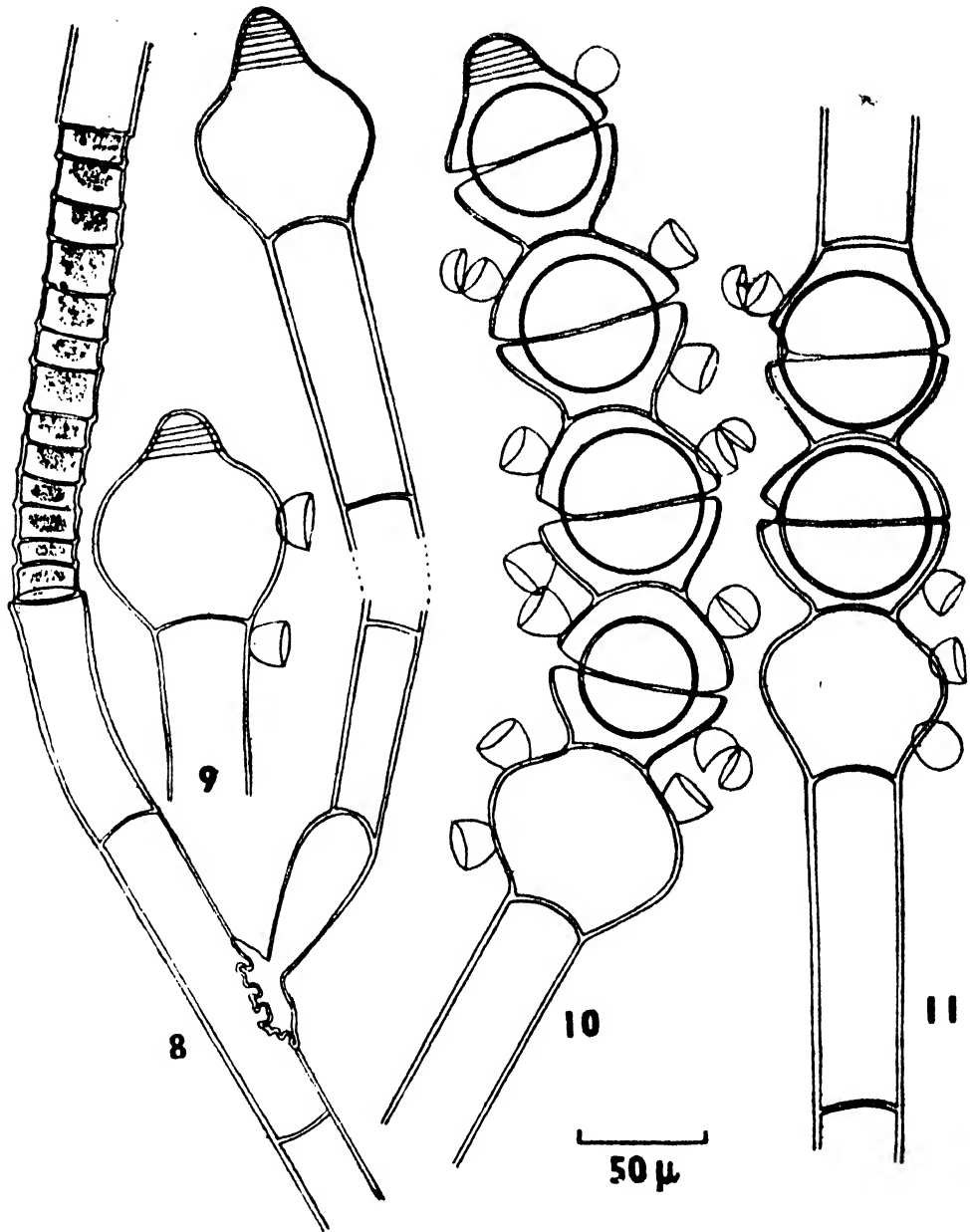
Distribution : Australia and China.

This form should, however, be compared with *Oe. keralense* Erady & Rajappan (1958) which was also described from Kerala. *Oe. keralense* is said to differ from *Oe. perspicuum* in having more globose and very slightly compressed oogonia, oospores filling the oogonia to a much greater extent than in the type, the idiandrosporus nature of the plants and the occurrence of nannandria occasionally on suffultory cells or even on the vegetative cells. The shape of the oogonia and the extent to which the oospores are filling the oogonia (a feature one of degree) show considerable variation in the same material, sometimes in the same filament, and hence may not serve as a specific criterion to distinguish the species.

Hirn (1900) in his original description of the type itself mentions "... (?) idiandrosporus ...", thus hinting its possibility, although the latter authors like Tiffany (1930) and Gemeinhardt (1939) have omitted this 'doubted' character in their monographs. The finding of the definite idiandrosporus nature of this present form and of *Oe. keralense* naturally clears the doubt of Hirn and adds to the original description a definite character as well as the occurrence of nannandria on or near oogonia or rarely on vegetative cells as observed in the present form and in *Oe. keralense*. An emended description of this species including all these features is given above.

In *Oe. keralense*, the antheridia are said to be "... antheridium internal, antheridial cells 2, lower cells smaller, sterile stipe cell absent, antherozoid one from each cell ...". In *Oe. perspicuum*, from which *Oe. keralense* is said to differ, the nannandria are unicellular. It is, however, not clear whether in *Oe. keralense* the unicellular nannandria produce two sperms or the nannandrium is two celled each functioning as an antheridium. If the latter is the case, as described, then the two celled antheridia alone separate *Oe. keralense* from *Oe. perspicuum* and at best, the former may be regarded as a variety of *Oe. perspicuum*.

The present authors do not agree with Hirn, (1900), Tiffany (1930) and Gemeinhardt (1939) in treating *Oe. dioicum* Carter in West & West (1901) as a synonym of *Oe. perspicuum*, since the former is dioecious-macrandrous with a superior division (West & West, 1901, Bot. Tidskr. 24 : 175, fig. 42) while *Oe. perspicuum* is dioecious-nannandrous with a median division.



Figs. 8-11. *Oedogonium perspicuum* Hirn Char. emend. Venkataraman and Natarajan. 8, a female filament epiphytic on an androsporangial filament; 9, a terminal oogonium (note the dwarf male on the suffultory cell); 10, a series of six oogonia terminally disposed; 11, a series of three intercalary oogonia (note the extent of variation in the oospores filling the oogonia in figs. 10 and 11).

Oedogonium perspicuum var. *keralense* (Erady & Rajappan) Comb. nov. (Syn. *Oe. keralense* Erady & Rajappan 1958. Kew Bull. Nr. 1, pp. 53-56, figs. 1-8).

Characters as in the species; mature dwarf males obovoid, two celled; antheridium internal, antheridial cells 2, lower cell smaller, antherozoid one from each cell.

Reported by Erady & Rajappan (1958) from shallow waters in the paddy fields at Mayyanad, Kerala State.

Cellula mascula nana obovoidea, bicellularis; antheridiis interioribus; ceterum ut in typo.

5. *Oedogonium longipilum* Jao. Jao, 1937, p. 304, t. II, figs. 14-16.

Dioecious, macrandrous; Oogonia 1, rarely 2, terminal ellipsoid, operculate, division supreme, operculum usually deciduous; Oospore of the same form as the oogonia and quite filling the same, spore wall smooth, antheridia 1-9, terminating the filament; sperm 1; basal cell not elongate, tumid; terminal cell extended into long hyaline seta.

Vegetative cells $7.6-11.4 \times 19-26.6\mu$; Oogonia $22.8-26.6 \times 26.6-30.4\mu$; Oospores $19.0-22.8 \times 22.8-26.6\mu$; antheridia $7.6-9.5 \times 11.4\mu$; basal cell $11.4 \times 19\mu$.

Habitat : Attached to the aquatic plants, Kottarakara, Kerala, January 15, 1959.

Distribution : Szechwan, China.

6. *Oedogonium armigerum* Hirn forma *major* form nov.

Dioecious, nannandrous; oogonium 1, subglobose, poriferous, pore superior; oospore globose, nearly filling the oogonium, outer layer of the spore wall echinate; dwarf male slightly curved on suffultory cell. stipe 2-4 celled sometimes; antheridium exterior; suffultory cell enlarged (fig. 12).

Vegetative cells $11.4-15.2 \times 64.6-114\mu$; suffultory cell $19 \times 76\mu$; oogonia, $38-45.6 \times 45.6-57\mu$; oospores $34.2 \times 34.2\mu$; dwarf male stipe $11.4 \times 34.2-41.8\mu$ antheridia $9.5 \times 15.2\mu$.

Dioicum, nannandrum; oogoniis singulis, subglobosis, poro superiore apertis; oosporis globosis, oogonia vix complentibus, episporio echinato; cellulis suffultoriis tumidis; nannandribus paullulum curvatis, in cellulis suffultoriis sedentibus, stipite interdum bi- vel tricellularis.

This form is characterized by echinate oospore wall and swollen suffultory cells. The present form is much bigger than the type and hence it is treated as a new form.

Habitat : Epiphytic on aquatic plants, Kottarakara, Kerala, January 15, 1959.

Distribution : The type species was reported from Brazil and forma *tenuis* Singh was from Gorakhpur, U.P., India.

Typus : Randhawa Collection OKI-160, Indian Agricultural Research Institute, New Delhi, India.

7. *Oedogonium cyathigerum* Wittrock var. *unicellulares* var. nov.

Dioecious, nannandrous, idiandrosporus; oogonia 1, subovoid to ellipsoid-ovoid, poriferous, pore superior; oospore same form as the oogonia, filling it, outer layer of the spore wall smooth inner layer with 16-20, sometimes anastomosing longitudinal ribs, dwarf males unicellular, globose to obovoid, on oogonium; antheridium interior, sperm 1 (figs. 13, 14).

Vegetative cells $38-45.6 \times 91.2-133\mu$; oogonia $68.4-72.2 \times 83.6-91.2\mu$; oospores $64.6-68.4 \times 72.2-72\mu$; androsporangia $30.4-34.2 \times (7.6) 11.4-19\mu$; dwarf males $15.2-19 \times 15.2-19\mu$.

Dioicum, nannandrium, idiandrosporum; oogoniis singulis, suboboviformibus vel oviformi-ellipsoideis, poro superiore apertis; oosporis forma oogoniis similibus et ea complentibus, episporia laevi; mesosporio longitudinaliter costato, costis continuis vel raro anastomosantibus, in medio oosporae circa 16-20; nannandribus unicellularibus, globosis vel oboviformibus, in oogoniis sedentibus; antheridiis interioribus.

This form can be readily distinguished from the type and the other varieties by its broader vegetative cells and globose to obovoid unicellular dwarf males as against the goblet-shaped dwarf males in others. The suffultory cell is not always swollen and it is not uncommon to find globose oospores also as in a form of *Oe. cyathigerum* reported by Fritsch & Rich (1924) from Africa.

Habitat : Free-floating, Kottarakara, Kerala, January 15, 1959.

Typus : Randhawa Collection OKI-161, Indian Agricultural Research Institute, New Delhi, India.

8. *Oedogonium spirale* Hirn. Hirn, 1900, p. 201, t. XXXIII, fig. 206; Tiffany, 1930, p. 123, t. XLIV, figs. 427, 428.

Dioecious, nannandrous, idiandrosporus; oogonium 1, subglobose or obovoid globose; poriferous, pore median; oospore globose or subglobose, not completely filling the oogonium; oospore wall double, the outer wall with 4-8 spiral ribs, anastomosing, united at the poles, inner wall smooth; androsporangia 1-4; dwarf male a little curved, near the oogonium; antheridium exterior, sperm 1 (fig. 15).

Vegetative cells $30.4 \times 60.8-133\mu$; oogonia $53.2 \times 64.6\mu$; oospores $45.6 \times 53.2\mu$; androsporangia $19-22.8 \times 11.4-15.2\mu$; dwarf male stipe $15.2-19 \times 57-60.6\mu$; antheridia $11.4 \times 15.2\mu$.

Habitat : Free-floating along with *Oe. cyathigerum* var. *unicellulares* var. nov. Kottarakara, Kerala, January 15, 1959.

Distribution : Ceylon, China, India (var. *acutum* West & West; var. *majus* Singh) Java, Latvia and United States of America.

9. *Oedogonium capillare* (Linn) Kütz. Hirn, 1900, p. 112, t. XI, fig. 58; Tiffany, 1930, p. 80, t. XVIII, figs. 164, 165; Gemeinhardt, 1939, p. 120, fig. 109.

Dioecious, macrandrous; oogonium 1, same as the vegetative cells in diameter; cylindrical to sub-cylindrical, poriferous, pore superior; oospore globose or rarely cylindric-globose, filling the oogonium or not, deep ochre in colour; spore wall smooth; antheridium 1-4, often alternating with the vegetative cells; sperms 2, by a horizontal division; basal cell elongate (Figs. 16-18).

Vegetative cells (female) $41.8-45.6 \times 57-64.6\mu$; vegetative cells (male) $34.2-41.8 \times 45.6-76\mu$; oogonia $41.8-45.6 \times 34.2-57\mu$; oospores $38 \times 30.4-38\mu$; antheridia $34.2-38 \times 7.6-11.4\mu$.

Habitat : Free-floating, Padmanabhapuram, Kerala, January 14, 1959.

Distribution : Africa, Austria, Denmark, Finland, France, Germany, Italy, India, Latvia, Russia, Spain, Sweden and United States of America.

10. *Oedogonium multisporum* Wood. Hirn, 1900, p. 232, t. XXXIX, fig. 239; Tiffany, 1930, p. 131, t. XLVI, figs. 450, 451.

Dioecious, nannandrous; oogonium 1-3, subglobose to subobovoid, pore superior; oospore globose, spore wall smooth; dwarf male a little curved or erect on oogonium; antheridium exterior, 1-4 (fig. 19).

Vegetative cells (7.6) $11.4-15.2 \times 19-87.4\mu$; oogonia $26.6-36 \times 26-36$ (38) μ ; oospores $26.6-32 \times 26.6-32\mu$; dwarf male stipe $11.4 \times 26.6-32\mu$; antheridia $7.6-11.4 \times 7.6-9.5\mu$.

Habitat : Free-floating, Krishnapuram, Kerala, January 13, 1959.

Distribution : China, India and England.

This form has much longer vegetative cells than in the type thus approximating those of *Oe. multisporum* var. *magnum* Ackley but agrees with the type in the measurements of the other parts.

11. *Oedogonium lageniforme* Hirn. Hirn 1900, p. 291, t. XIII, fig. 68; Tiffany, 1930, p. 168, t. XX, fig. 187; 1937 II, p. 35, t. X, figs. 134-136; Gemeinhardt, 1939, p. 346, fig. 425.

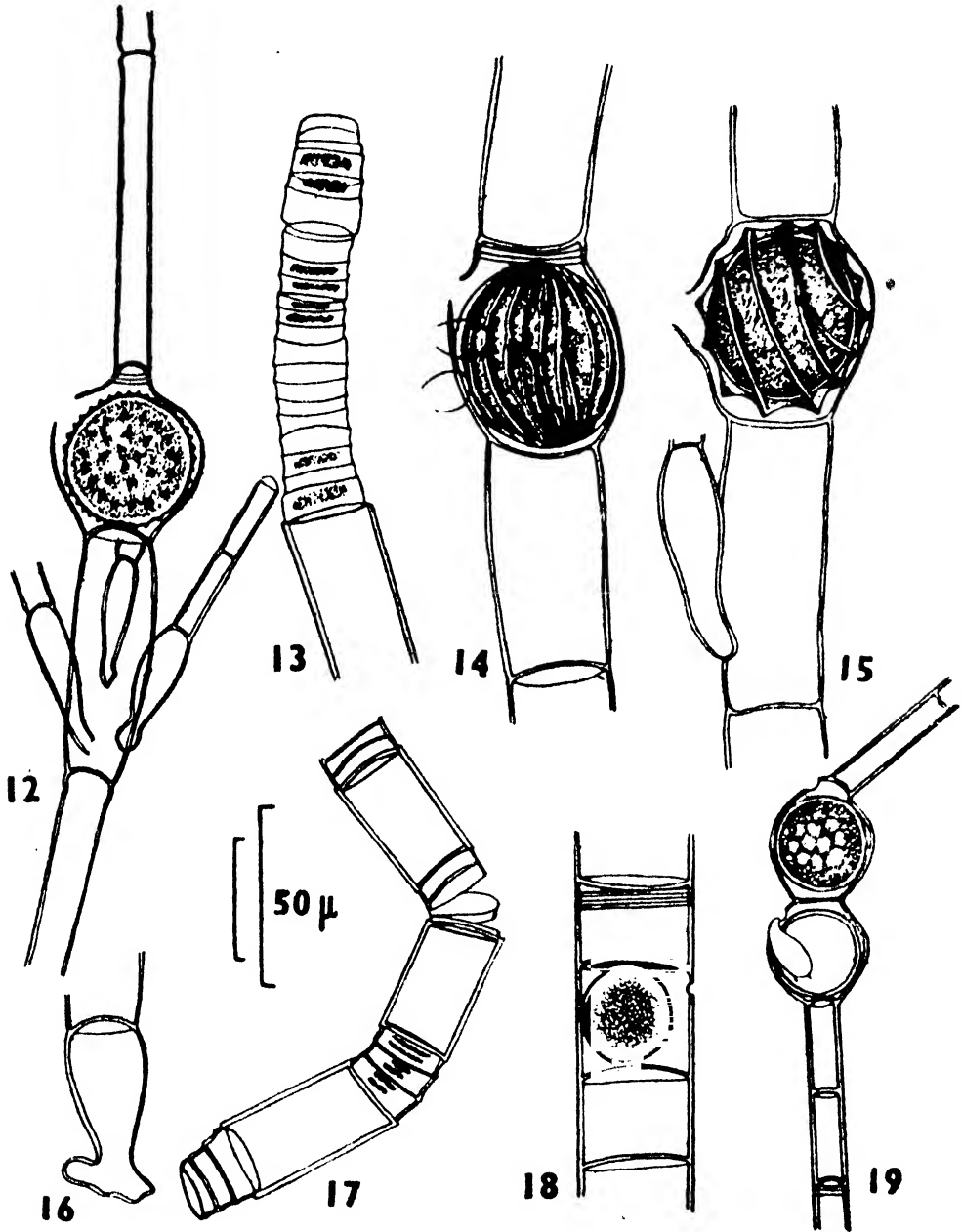


Fig. 12-19. *Oedogonium armigerum* forma *major* form nov; 13-14. *Oe. cyathigerum* var. *unicellulare* var. nov; 15. *Oe. spirale* Hirn.; 16-18. *Oe. capillare* (Linn) Kütz. 19. *Oe. multisporum* Wood.

Dioecious, macrandrous; oogonium 1, obpyriform, ellipsoid or subglobose ellipsoid, in section usually binodulose, inferiorly inflated, poriferous, pore superior; oospore globose to ellipsoid, in the lower inflated portion spore wall smooth.

Vegetative cells $11.4-15.2 \times 38-89\mu$; oogonia $22.8-34.2 \times 38-57\mu$; oospores $26.6-30.4 \times 30.4-38\mu$.

This is an imperfectly known species but can be readily distinguished by its binodulose oogonium which is inferiorly inflated. This form must be compared with *Oe. ellipsoideum* Jao where the oogonium is superiorly inflated. The male plants of this form have been recorded by Tiffany from the collections in Puerto Rico by N. Willy. However, only the female plants of this form are found in the present collections and that too quite sparse.

Habitat: Epiphytic on aquatic plants along with *Bulbochaste intermedia* and *B. varians* var. *subsimplex*. Very rare. Krishnapuram, Kerala, January 13, 1959.

Distribution: Brazil, China and N. America.

12. *Oedogonium paucocostatum* Transeau. Transeau, 1914, p. 300, t. XXVIII, fig. 5; Tiffany, 1930, p. 98, t. XXXII, fig. 277; Gemeinhardt, 1939, p. 169, fig. 178.

Diocious, macrandrous; oogonium 1, ellipsoid, operculate, division superior; oospore same form as the oogonium, filling the oogonium, outer and inner spore wall smooth, middle layer with 14-20 longitudinal ribs; antheridia 2-8 seriate, sperms 2, by horizontal division (figs. 20-21).

Vegetative cells $19-26.6 \times 7.6-15.2\mu$; oogonia $57-76 \times 7.6-9.5\mu$; oospores $47.5-57 \times 57-85.5\mu$; antheridia $15.2-22.8 \times 7.6-11.4\mu$.

Habitat: Free-floating, Kottarakara, Kerala, January 15, 1959.

Distribution: China and United States of America.

13. *Oedogonium hians* Nordstedt and Hirn. Hirn, 1900, p. 227, t. XXXVIII, fig. 233; Tiffany, 1930, p. 149, t. LVIII, figs. 570-571; 1937 II, p. 70, t. XXVIII, figs. 449-450; Gemeinhardt, 1939, p. 304, fig. 363.

Diocious, nannandrous, gynandrosporus; oogonia 1-2, globose to subglobose, operculate, division superior, wide; suffulatory cell enlarged; oospore globose to slightly subglobose, filling the oogonium, spore wall smooth; androsporangia 1-2, subepigynous, dwarf male slightly curved on suffulatory cells; antheridium 1, exterior; vegetative cells slightly capitellate; terminal cell obtuse (fig. 22).

Vegetative cells $11.4-15.2 \times 22.8-49.4\mu$; suffulatory cell $30.4-34.2 \times 45.6-76\mu$; oogonia $34.2-38 \times 30.4-41.8\mu$; oospores $30.4-36.1\mu$; androsporangia $11.4 \times 15.2-19\mu$; dwarf male stipe $7.6 \times 38\mu$; antheridia $7.6 \times 3.8-5.7\mu$.

This form is characterised by the swollen suffulatory cells, wide operculum and slightly capitellate vegetative cells.

Habitat: Free-floating, Krishnapuram, Kerala, January 13, 1959.

Distribution: Brazil, India, United States of America.

14. *Oedogonium howardii* West. Hirn, 1900, p. 16, t. III, fig. 9; Tiffany, 1930, p. 101, t. XXXIII, figs. 293-294; 1937 II, p. 49, t. XX, figs. 290-291; Gemeinhardt, 1939, p. 180, fig. 190.

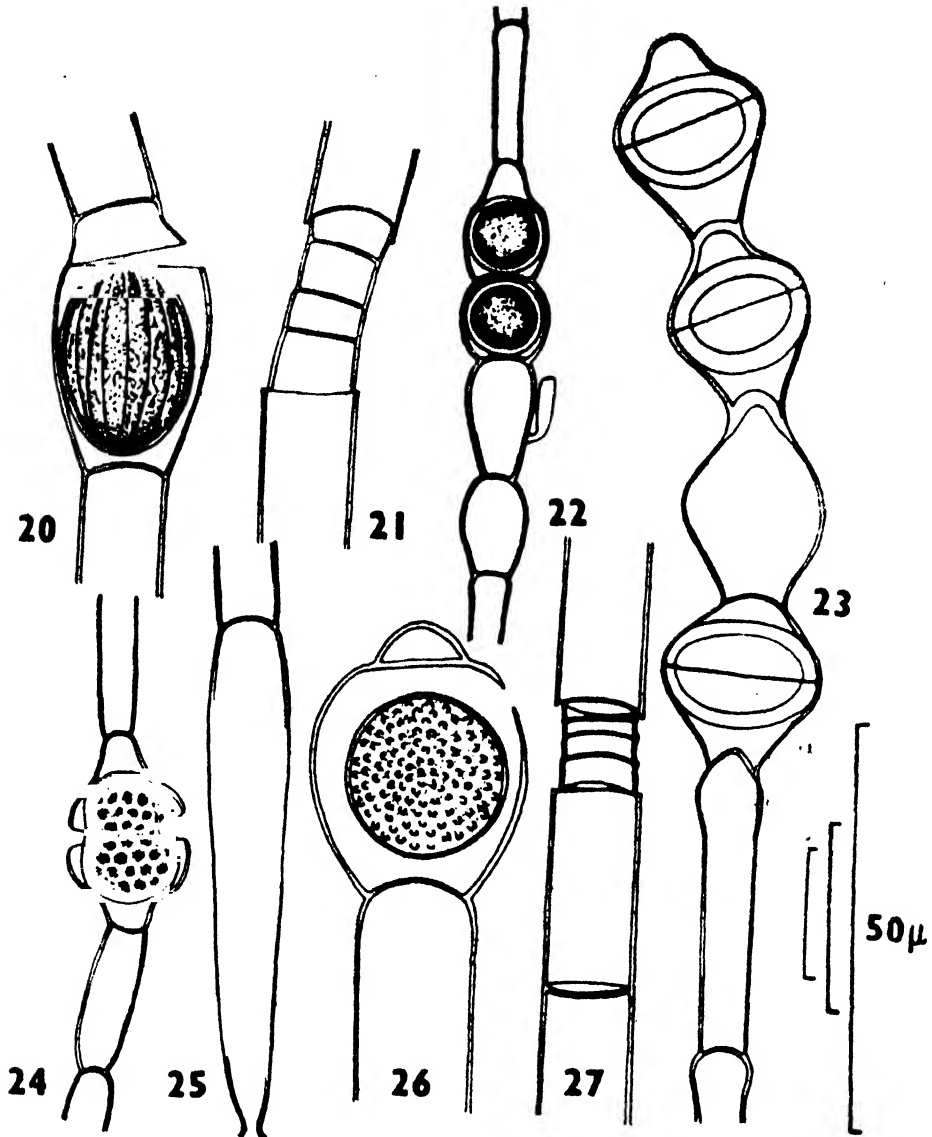
Diocious, macrandrous; oogonia 1-2, globose or subglobose, operculate, division median; oospore same size as the oogonia, completely filling the oogonium; antheridia 1-12 seriate; vegetative cells distinctly capitellate; basal cell spherical to hemispherical.

Vegetative cells $7.6-11.4 \times 15.2-26.6\mu$; oogonia $22.8-26.6 \times 19-26.6\mu$; oospores $22.8-24.9 \times 20.9-22.8\mu$; antheridia $7.6-9.5 \times 7.6-11.4\mu$; basal cell $11.4-15.2 \times 11.4-19\mu$.

Habitat: Freshwater, Kottarakara, Kerala, January 15, 1959.

Distribution: Barbadas, China, India and United States of America.

15. *Oedogonium pusillum* Kirchner. Hirn, 1900, p. 299, t. XXIV, fig. 125; Tiffany, 1930, p. 161, t. XXXIV, fig. 316; 1937 II, p. 82, t. XIX, fig. 287; Gemeinhardt, 1939, p. 331, fig. 400.



Figs. 20-21. *Oedogonium paucocostatum* Transouu; Fig. 22. *Oe. hians* Nord & Hirn; Fig. 23. *oe. tapeinosporum* f. *fowlingense* Jno; Fig. 24. *Oe. pusillum* Kirchner; Fig. 25-27. *Oe. hians* Skuja.

Oogonium 1, subbiconic-ellipsoid or subbiconic-globose, operculate, division median, wide; oospore globose to ellipsoid, slightly constricted in the middle, spore wall smooth; apical cell obtuse; basal cell subhemispherical (fig. 24).

Vegetative cells $3.8-5.7 \times 12.04-38\mu$; oogonia $13.76 \times 20.64\mu$; oospores $12.04 \times 15.2\mu$.

Male reproductive structures of this form were not seen, but this species is readily distinguished by its medianly constricted oospore and the wide operculum.

Habitat : Epiphytic on aquatic plants, Kottarakara, Kerala, January 15, 1959.

Distribution : Africa, Austria, Burma, Brazil, Europe, India, Sumatra and United States of America.

16. *Oedogonium poecilosporum* Nordstedt & Hirn. Hirn, 1900, p. 298, t. XXIII, fig. 124; Tiffany, 1930, p. 168, t. XXXIV, fig. 305; 1937 II, p. 84, t. XIX, fig. 286; Gemeinhardt, 1939, p. 347, fig. 426.

Oogonium 1-2, depressed-globose, operculate, division median, wide; oospore depressed-globose, nearly filling the oogonium, spore wall smooth; basal cell sub-hemispherical; terminal cell obtuse.

Vegetative cells $5.7-7.6 \times 41.8-60.8\mu$; oogonia $22.8-26.6 \times 26.6-38\mu$; oospores $19-24.7 \times 19.0-22.8\mu$.

Habitat: Epiphytic, Kottarakara, Kerala, January 15, 1959.

Distribution: Africa and United States of America.

17. *Oedogonium tapeinosporum* forma *foulingense* Jao. Jao, 1937, p. 307, t. III, figs. 25-28.

Oogonia 1 to many, depressed-globose or pyriform or pyriform-globose, operculate, division median or slightly supramedian, narrow; oospore depressed globose, not filling the oogonium longitudinally; spore wall smooth (fig. 23).

Vegetative cells $5.16-6.8 \times 20.64-29.24\mu$; oogonia $17.2-20.64 \times 20.64-24.08\mu$; oospores $15.2-19 \times 12.04-15.48\mu$.

This form has slightly broader dimensions than the type.

Habitat: Epiphytic on aquatic plants, Krishnapuram, Kerala, January 13, 1959.

Distribution: China and India (Fyzabad, U.P.).

18. *Oedogonium Ilsteri* Skuja. Skuja, 1934, p. 59, fig. 80; Gemeinhardt, 1939, p. 362, fig. 449.

Diocious, macrandrous; oogonium terminal or intercalary, 1-2, pyriform, poriferous, pore superior; oospore globose, not filling the oogonium, outer spore wall thin and smooth, middle layer scrobiculate; suffultory cell not or very slightly swollen; antheridia 2 to many seriate (figs. 25-27).

Vegetative cells (female) $19-38 \times 68.4-190\mu$; vegetative cells (male) $19-26.6 \times 45.6-144.4\mu$; oogonia $60.8 \times 64.6-72.2\mu$; oospores $45.6 \times 45.6\mu$; antheridia $19-22.8 \times 7.6-11.4$.

Habitat: Epiphytic on aquatic plants, Kottarakara, Kerala, January 15, 1959.

Distribution: Europe.

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AN EXPERIMENTAL STUDY OF THE OPTIMUM SALINITY FOR THE GROWTH OF THE BENTHIC BLUE GREEN ALGA, *OSCILLATORIA SPLENDIDA*, GREVILLE OF BRACKISHWATER PONDS

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(Communicated by B. S. Bhimachar, F.N.I.)

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ABSTRACT

Pot culture experiments were conducted to ascertain the optimum range of salinity for the growth of the benthic blue-green alga, *Oscillatoria splendida* found in brackishwater fish ponds. The experimental methods are described in detail. The results of the experiments show that a salinity ranging between 5 parts per thousand and 17 parts per thousand is most favourable for the growth and reproduction of this alga during the summer months.

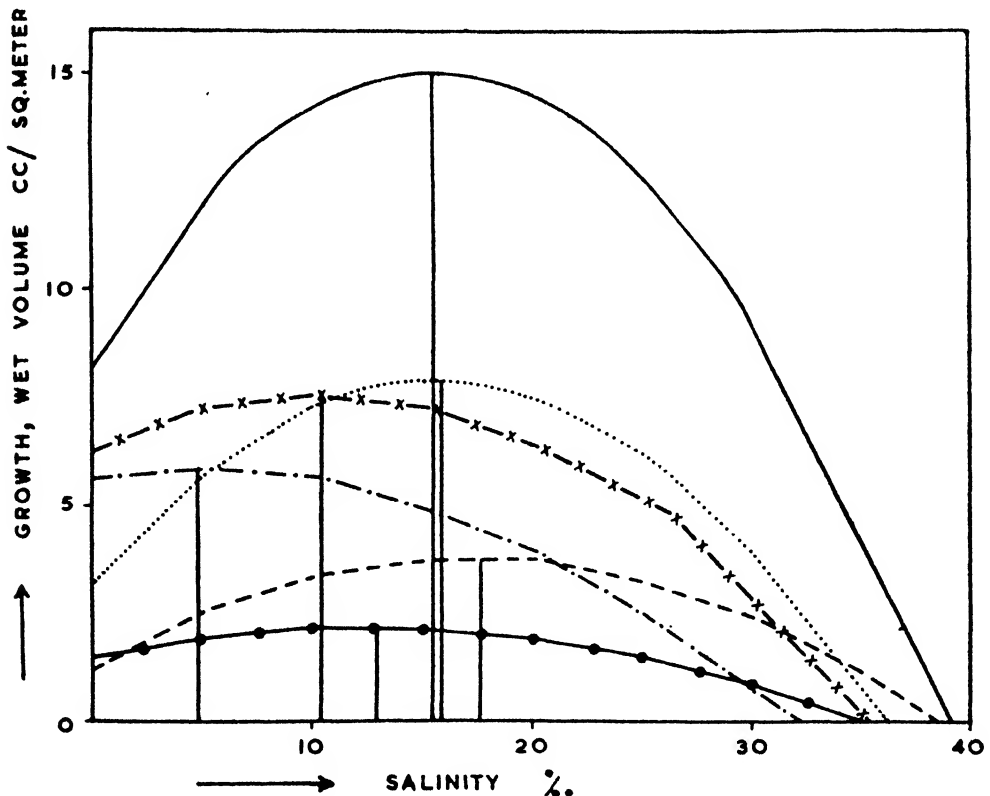
INTRODUCTION

Benthic algae form the main food of cultivated fishes like the Mullet and Milk fish in brackishwater ponds (Schuster, 1952; Pillay, 1954) and therefore the enhancement of the growth of these algae by the provision of optimum conditions for their growth and multiplication forms a very important aspect of fish culture in such ponds. General field observations on the role of certain environmental factors have been conducted by different workers. A suitable salinity of the water of the fish ponds has been considered as one of the essential environmental requirements for the growth of benthic algae (Frey, 1947; Carbine, 1948; Rabanal *et al.* 1951a). Schuster (*loc. cit.* p. 19) has observed that a higher salinity between 15-45 parts per thousand is preferable for their growth. According to Rabanal *et al.* (1951a) the salinity tolerance for the blue green algae is between 10 and 40 parts per thousand. Rabanal (1949) has also reported that brackishwater is better than highly saline marine water or freshwater for the growth of blue green algae. Pillai (1954), however, has observed in the case of a blue green alga, *Phormidium tenue*, that a high salinity even upto 85 parts per thousand may be suitable for their normal growth. Unlike blue greens, green algae have been observed to grow better in experimental fish ponds having lower salinities, ranging between 9.0 parts per thousand and 22.2 parts per thousand than in those having salinities ranging between 20.6 parts per thousand and the 34.2 parts per thousand (Rabanal *et al.* 1951b). While above observations are of great interest, critical experiments to ascertain the salinity requirements of different species of algae, which would be of basic importance in evolving scientific fish cultural methods, do not appear to have been undertaken so far. In connection with the investigations on brackishwater fish culture in the estuarine areas of West Bengal, experimental studies have been undertaken to determine the optimum environmental requirements of important species of algae. This paper embodies the results of experiments conducted to elucidate the optimum salinity required for the growth of the blue green alga, *Oscillatoria splendida* Greville, which is a common food item of grey Mullet, specially in their fry and fingerling stages.

EXPERIMENTS

The experiments were conducted in semi-field conditions in large earthen pots of a uniform diameter of about 65 cm. at the mouth. Mud collected from the same locality and of the same consistency was used as the substrata, after exposing the

whole mud for an hour to a temperature of about 80°C to kill any algal spores, etc. present in it. The surface area of the mud in the pots measured about 0.2 sq. meter. Since a shallow depth of water is considered favourable for the growth of blue green algae, 6 to 7 cm. depth of water was maintained in the pots. Boiled tap water was used, after cooling, to prepare water of different salinities for the experiments. In each experiment a series of 9 identical pots with water of salinities ranging between 0 part per thousand and 40 part per thousand at intervals of 5 part per thousand was used. A variation of ± 2 parts per thousand was observed to occur in the experimental pots on account of evaporation or accidental admixture of rain water. The required algae for the experiments were cultured in the laboratory in petri-dishes on mud surface. About one sq. cm. of the algal layer (not free from bacteria or diatoms) was inoculated in each of the pots on the mud surface. The pots were kept under the open sky with necessary protection from disturbances by birds, etc. The temperature of water in the pots was noted daily and the water level and salinity adjusted as required to compensate the effect of evaporation. When the algal layer had completely covered the mud surface in the pots, showing good growth, it was dislodged by mechanical means; then siphoned out through a rubber tubing and passed through a fine sieve (No. 100). The mud particles attached to the algae were carefully washed out and the algae removed from the sieve to a beaker with water. They were subsequently filtered over a known volume of glass-wool and the wet volume measured by water



TEXT-FIG. 1.

The inflection points on the regression curves showing the corresponding salinity for the maximum growth of algae.

displacement after removing the superfluous water by means of blotting paper. By deducting the volume of glass-wool, the volume of the algae was obtained.

Six series of experiments, with replications except in two cases, were conducted at different times during summer* months when the temperature of water in the pots varied between 27°C and 38°C.

Second degree parabolas of the intensities of algal growth as observed in different salinities in the six experiments were determined by the method of least squares and these are delineated in Text Fig. 1. The regression equations obtained are as follows :

Expt. No.	Regression Equation
**1	$Y = 1.1941 + 0.2899x - 0.0083x^2$
2	$Y = 8.2804 + 0.8783x - 0.0282x^2$
3	$Y = 1.4738 + 0.1144x - 0.0045x^2$
4	$Y = 6.2701 + 0.2494x - 0.0121x^2$
5	$Y = 3.1440 + 0.5960x - 0.0190x^2$
**6	$Y = 5.6480 + 0.0732x - 0.0080x^2$

$Y =$ growth c.c./sq meter and $X =$ salinity, parts per thousand

The points of inflection of these curves (obtained by equating $\frac{dy}{dx} = 0$) are not the same, which would suggest that any salinity within a range is favourable for their growth. It is clear from the position of the inflection points marked by perpendicular lines in Text Fig. 1 that a salinity ranging between 5 parts per thousand and 17 parts per thousand is the most favourable for the growth of this alga.

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*The winter variation of temperature, between 17°C and 28°C has been observed to be not conducive for the growth of this species.

**These two experiments were without replication.

**PAHUDIOXYLON BANKURENSIS GEN. ET. SP. NOV.—A FOSSIL WOOD
FROM THE MIOCENE BED OF BANKURA DISTRICT.
WEST BENGAL, INDIA**

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(Received May 21: read August 7, 1959)

ABSTRACT

A fossil dicotyledonous wood collected from Bankura Damodar Railway-cutting, south of 18/7 railway mile post, north of Gangabandh village, is reported here. Its age is possibly Upper Miocene. It appears to be new to science and is named *Pahudioxylon bankurensis* Gen. Et. Sp. Nov.

INTRODUCTION

While mapping Bankura district of West Bengal, Shri A. L. S. Hunday, Geological Survey of India, Calcutta, collected a fossil wood specimen during his 1952-53 field survey. The exact locality from which this specimen was collected has been given by him as Bankura Damodar River Railway-cutting, south of 18/7 railway mile post, north of Gangabandh village (sheet 73-M/7), Bankura district. The fossil bears G.S.I. No. P2/126.

The preservation of the fossil is fairly good. It has a variegated colour which ranges from creamy white to reddish brown with sparkling yellow or scarlet red patches at irregular intervals. In size, the specimen is about 36 cm. long and 15 cm. wide, and somewhat round in shape. Examination by hand lens shows that the specimen is a part of a fairly large trunk. It is from a portion neither very close to the centre of the trunk nor from near the bark.

As to the age of the fossil the Geological Survey of India says, "It seems fairly safe to assume that the Bankura and Garbata specimens may belong to the Miocene, and possibly the Upper Miocene".

**ANATOMY OF THE FOSSIL—G. S. I. NO. P2/126 AND ITS COMPARISON
WITH THE SECONDARY XYLEM OF LIVING PERENNIALS**

A. Macroscopic study

It is a porous wood indicating its affinity with the living dicotyledons. The structure is typically diffuse-porous.

Growth rings are distinctly visible to the naked eye, 3-6 rings per cm. At certain places, growth marks have been noticed to run in an undulating fashion. The rate of growth varies considerably.

Vessels are easily visible to the eye. They are usually solitary and sometimes in radial multiples of 2-3 (Text-fig. 1). They are generally round to oval in shape. Tyloses are absent but gummy deposits occasionally fill up some vessels. The distribution of vessels is more or less uniform throughout the specimen.

Fibres are individually indistinct, although collectively they show up prominently against the other tissues.

Parenchyma cells are clearly visible to the naked eye. In fact, the most conspicuous structure is its parenchyma distribution (Text-fig. 1). Firstly, they are in initial bands demarcating the growth rings. This structure is a prominent and constant feature of the wood. Secondly, they are paratracheal showing aliform

to aliform-confluent distribution. Often they are in thick patches surrounding a batch of vessels and forming an undulating outline. The aliform type is characteristic and mostly appears as almond-shaped (Pl. II, Figs. 1, 3).

Rays are not very clear to the eye but distinct under a hand lens. They are more or less evenly distributed, showing up as fairly wide, white lines on the end surface. On the tangential surface they appear to be very low.



TEXT-FIG. 1.

Diagrammatic sketch to show general pattern of parenchyma distribution in the fossil, $\times 11$.

Amongst the gross anatomical features of the fossil, the following points are of importance :

- (i) It is a diffuse-porous wood,
- (ii) Its vessels are evenly distributed,
- (iii) The parenchyma forms distinct aliform to aliform-confluent structure. Although each growth ring may show some variation in the structural pattern of these parenchyma cells, yet there is no doubt that they are the major types of parenchyma distribution in the wood.
- (iv) Rays are fairly wide and low.

From a comparative study of the wood samples at our disposal as well as from some published literature on the secondary xylem of living dicotyledons, namely, Anon. (1953), Chalk (1932-39), Lecomte (1926), Metcalfe and Chalk (1950), Moll and Janssonius (1914), Normand (1950), Pearson and Brown (1932), Reyes (1938), it appears that the fossil under investigation may belong to *Anacardiaceae*, *Combretaceae*, *Leguminosae*, *Moraceae* and *Sapindaceae*. Further elimination amongst these families is, however, possible only on the basis of microscopic structure.

B. Microscopic structure

Growth rings : The conspicuous concentric marks at narrow to wide intervals are due to initial parenchyma cells.

Vessels are moderately large to large. Their distribution shows 2-3 per mm². There is very little difference in size between the early and the late pores. Occasionally one may come across some small pores near the growth marks but this is not a constant feature. Vessels are fairly thick-walled. Perforation plates are simple, horizontal to slightly oblique. Inter-vessel pits are alternate, rather small, not so crowded. The vested nature of the pit is indicated under high power (Pl. II, Fig. 6). Vessel-ray and vessel-parenchyma pits are somewhat similar to inter-vessel pits.

Fibres are fairly well-preserved at places but not so well as the parenchyma cells. They are slightly angular to round in cross section and show somewhat radial alignment. They are moderately thick-walled, non-septate and non-libriform.

Parenchyma cells are of two types : (a) The initials are 2-3 cell thick demarcating the seasonal growth. In shape they are nearly rectangular in cross section ; (b) Paratracheals show up prominently. Those that are adjacent to the vessels are rather elongated adjusting themselves to the outer walls of the vessels. Those that are far away from vessels are rectangular to roundish in shape. The general structural pattern they produce is mostly aliform-confluent. In fact, this is the most conspicuous structure that characterizes the fossil. The peculiarity of the fossil appears to be aliform-confluent type of distribution of parenchyma cells in which 3-4-5 to 5 or even 6 vessels or vessel-pairs are linked up. In the longitudinal section there is no tendency for the parenchyma cells to be arranged in storeyed structure. There are, however, many parenchyma cells which are chambered.

Rays are fairly prominent, uniseriate to triseriate, mostly 2-3 seriate and homogeneous ; uniseriate rays are 4-10 cells in height. Multiseriate rays are upto 25 cells and 300 μ in height. They show a distinct tendency to an arrangement in echelon and may give at places the impression of faint ripple-marks. The individual cells are mostly round to slightly oval and moderately thick walled.

The next stage is to compare these anatomical details of the fossil with those of the living members of the five families mentioned earlier in this paper. In the family *Anacardiaceae*, two genera *Mangifera* and *Semecarpus* show some similarity with the fossil in the cross-section but careful scrutiny reveals that they are different from the fossil in two respects, i.e. in conspicuous aliform parenchyma and the ray structure. There is also some resemblance between the fossil and the *Terminalias* of the *Combretaceae* and *Nephelium lappaceum* of the *Sapindaceae*. But on the basis of uniseriate rays and thin walled nature of ray cells, members of these two families can also be eliminated. Although the genus *Artocarpus* of the *Moraceae* may show some resemblance with the fossil in respect of the structure visible on the end surface yet it is evident that the unknown fossil is not an *Artocarpus* because there is neither initial parenchyma nor vested pits in the latter. We are now, therefore, left with timbers of the family *Leguminosae*.

In an investigation on a somewhat similar fossil wood from the eastern corner of Raniganj coal field of India, one of us (Chowdhury) has classified some members of the living *Leguminosae* which have paratracheal parenchyma cells. Taking the data from a table given in that paper, it appears that only a limited number of genera show affinity with the fossil under investigation and these are : *Afzelia*, *Albizia* (except *A. amara*, *A. coriaria*, *A. falcata*, *A. ferruginea*, *A. mollis* and *A. moluccana*), *Cylicodiscus*, *Desmodium tiliaefolium*, *Intsia*, *Macrolobium*, *Ougeinia*, *Pahudia*, *Parkia*, *Pithecolobium* and *Saraca*.

Now a good many of these woods can be eliminated by the structure of the rays visible on the tangential surface. For instance, in *Cylicodiscus* the rays are always 2-4 seriate while *Desmodium tiliaefolium* is ring-porous. The rays in *Parkia* and *Saraca* are heterogeneous. Again, in *Pithecolobium* they are uniseriate. *Macrolobium* has also exclusively uniseriate rays which are rather thin walled. Although *Ougeinia* has 2-3 seriate rays like the fossil yet it can be easily discarded because of constant presence of ripple-marks in it. We are thus now left with four genera,

namely, *Azelia*, *Albizzia*, *Intsia*, and *Pahudia*. Of these, the first three genera are so similar that they cannot at present be separated by the anatomical structure of their secondary xylem. They, however, differ from *Pahudia* in having septate fibres. The point to note here, is that the fossil under investigation has shown no septate fibre. It will, therefore, be seen that the G. S. I. No. P2/126 shows the greatest similarity with the genus *Pahudia*.

COMPARISON OF THE FOSSIL WOOD G.S.I. No. P2/126 WITH THE DICOTYLEDONOUS FOSSIL WOODS PREVIOUSLY REPORTED

A. Fossil from India

There are only a few leguminous fossil woods that have so far been reported from India. It was Gupta (1936) who for the first time showed affinities of a specimen from Burma to *Leguminoxylon burmense* Gupta (1936). Description of this fossil wood given by him does not match with the anatomy of the specimen under study here.

In 1953 Ramanujam reported *Albizzia* from the Tertiary rock of South Arcot. Again he reported (1954a) some Leguminous woods from the same locality. These two notes however, are not accompanied by photomicrographs nor by diagrams. It is therefore, not possible for us to say whether Bankura specimen matches with those reported by Ramanujam. Later, in 1954 he reported again two members of the family *Leguminosae* from near Pondicherry. They are *Caesalpinioxylon sitholeyi* and *Acacioxylon indicum*. The first specimen shows no clear growth marks. Its fibres are libriform to semi-libriform. The rays are 2-3 seriate but the shape of individual ray cell is not given. Under the rays he says there is "a definite tendency towards storied arrangement", but in another place he reports "there are no ripple marks". It will, therefore, be seen that the G.S.I. fossil wood No. P2/126 does not match with *Caesalpinioxylon sitholeyi*.

As regards *Acacioxylon indicum*, Ramanujam reports that it has faint growth marks, scanty vessels, and 2-5 seriate rays with individual cells round to oval and no ripple marks. Fibres are described as "typically septate" a feature which we have not come across so far in the living *Acacias*. In view of all these differences, it can be said that Bankura fossil is not similar to *Acacioxylon indicum*.

B. Fossil from outside India

Several fossil woods from outside India have been reported to show affinities with the woods of the family *Leguminosae*. At present there are 5 well established form genera. These are *Cassioxylon*, *Acacioxylon*, *Caesalpinioxylon*, *Leguminoxylon* (Edwards 1931) and *Cynometroxylon* (Chowdhury and Ghosh, 1946). Without going into the merit of the creation of these genera, we shall consider only those specimens which show some similarity with the fossil under investigation.

Among the *Acacioxylon*, only *A. antiquum* Schenk Krausel (1939), may be considered here. This specimen has banded parenchyma, making it entirely different from our fossil. Within the form genus *Caesalpinioxylon*, *C. quirogae* Schenk, *C. migiurtinum* Chia., *C. Ducis-Aprutii* Chia., *C. Zaccarini* Chia. (1953) and *C. mogadaense* Bour. (1950), show some general similarity with the fossil from Bankura in gross features. But *C. quirogae* Schenk has uniseriate rays and cannot therefore be matched with it. Chiarugi's three specimens are also different from our fossil, for *C. migiurtinum* has vertical gum ducts of traumatic type, *C. Ducis-Aprutii* has scanty parenchyma and less number of vessels per unit area while *C. Zaccarini* has diffuse parenchyma and 3-5 seriate rays. Again in *C. mogadaense* Bour, parenchyma cells are profusely developed and the rays are mostly uniseriate. It will, therefore,

be seen that none of the *Caesalpinioxylon* so far reported can be matched with G.S.I. fossil No. P2/126.

As regards *Leguminoxylon*, 3 species reported by Krausel, one by Schonfield (1947), and one by Boureau (1951) will be considered here. *L. acaciae* Kr. has diffuse parenchyma, and 1-2 seriate homogeneous rays and its fibres are entirely libriform. Thus, there is considerable difference from our fossil. In *L. Edwardsi* Kr., rays are uniseriate and homogeneous and there is scanty development of parenchyma cells. Again, in *L. albizziae* Kr., there are vertical gum ducts of traumatic type and the growth rings are demarcated by thick walled late fibres. Thus none of Krausel's specimen shows similarity with Bankura specimen. Now Schonfield's specimen *L. grossei* has paratracheal broken bands and rays are 2-3 seriate and 30-60 cells high. So this also can be eliminated. Finally, let us consider Boureau's specimens *L. Menchikoffii* from Eocene of Algeria. This fossil shows profuse development of parenchyma and has mostly 2-seriate rays which occasionally may be 4-seriate. It will, therefore, be clear that none of the eleven species of fossil leguminous woods compared here matches in all respects with our fossil marked G.S.I. P2/126.

NAME AND DIAGNOSIS

The fossil wood under investigation shows close affinities with the secondary xylem of the living genus *Pahudia* (*Leguminosae-Caesalpinioideae*). In our opinion the names *Leguminoxylon* Gupta (1936) and *Caesalpinioxylon* Schenk (1890) are likely to bring confusion because under these classes made by systematists, we have plants which show a great diversity of anatomical structure in their secondary xylem.

The generic name *Pahudioxylon* proposed here includes all *Pahudias* now known to Science. The fossil G.S.I. P2/126 is specifically named as *Pahudioxylon bankurensis*. Its generic and specific diagnoses are given below :

Genus : *Pahudioxylon* K.A. Chowdhury, S.S. Ghosh and M.H. Kazmi.
A diffuse-porous wood.

Growth rings : distinct and clear, 3-6 per cm.

Vessels : moderately large to large, clearly visible to the eye, solitary or in radial multiples 2-3, fairly thick-walled. Tyloses absent but gummy deposits sometimes present. Perforation plate simple, horizontal to slightly oblique. Intervessel pits rather small, alternate, not crowded, vestured (Pl. II, Fig. 6). Vessel-ray and vessel-parenchyma pits almost similar to inter-vessel pits.

Fibres : fairly distinct. In cross section round to angular, showing tendency for radial alignment. They are non-libriform and non-septate.

Parenchyma : prominent, visible to the eye. Two types are seen : (a) initial, 2-3 cells thick, nearly rectangular in cross section, demarcate the growth rings clearly ; (b) paratracheal forming aliform to aliform-confluent structure, more often aliform-confluent.

Rays : fairly clear to the eye but distinct under hand lens with a tendency for faint ripple marks, mostly 2-3 seriate, homogeneous. Individual cells fairly thick-walled, mostly round, rarely oval.

Species : *Pahudioxylon bankurensis* K. A. Chowdhury, S. S. Ghosh and M. H. Kazmi.

Vessels : more or less evenly distributed, very few to few, 2-3 per mm²., solitary or in radial multiples, 20 per cent in pairs, the rest solitary. Tangential diameter solitary vessels varies from 52-180 μ and radial 52-225 μ .

Fibres : no maceration was possible to obtain, hence length not known.

Parenchyma : two types (a) initials somewhat flattened, (b) paratracheal next to the vessels flattened to adjust themselves to the wall of vessels, far away from vessels rectangular to round ; non-storied, chambered with single crystal.

Rays : moderately broad, rather widely spaced, 2-3 seriate, 30-40 μ in width, 15-25 cells and 280 to 365 μ in height. Individual cells 12-16 μ in width.

SUMMARY

1. A dicotyledonous fossil wood from Bankura District, West Bengal, India, is described and recorded here. It bears the number G.S.I. P2/126.

2. An anatomical study of the fossil has been made. It shows affinities to living genus *Pahudia* of the *Leguminosae*.

3. Comparison of this fossil with those previously reported from India and outside, has been made and their possible affinities are discussed. The fossil G.S.I. P2/126 appear to be the first specimen of *Pahudioxylon* so far reported and it is named *Pahudioxylon bankurensis*.

4. The age of the fossil is believed to be Miocene, probably Upper Miocene.

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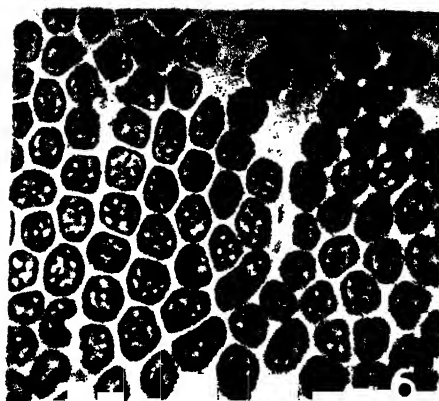
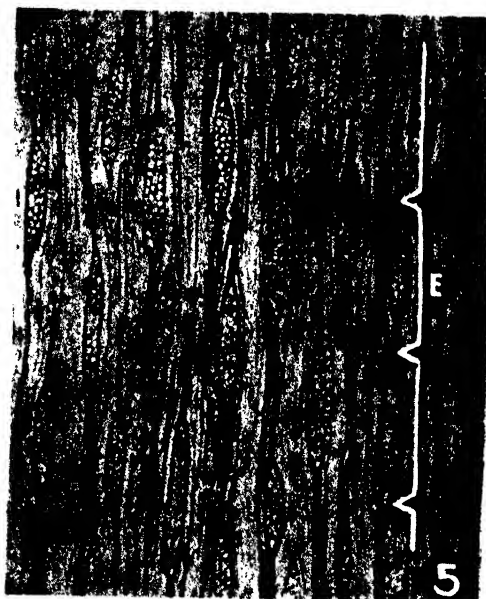
EXPLANATION OF PLATE

[No retouching has been done to any negative]

Plate No. II

Pahudioxylon bankurensis, Gen. Et Sp. Nov.

- Fig. 1. Transverse section showing distribution of vessels, rays (R), Parenchyma arrangement and growth marks (GM) $\times 10$.
- Fig. 2. Transverse section showing aliform (AF) and aliform confluent (AC) type of parenchyma, growth mark with initial parenchyma cells and fibers (F) $\times 30$.
- Fig. 3. Transverse section showing mainly aliform parenchyma (AF) and arrangement of fibres $\times 30$.
- Fig. 4. Tangential section showing shape and size of the rays and their composition $\times 100$.
- Fig. 5. Tangential section showing ray distribution. Note that some rays are arranged in echelon (E) $\times 63$.
- Fig. 6. Vestured nature of inter-vessel pits $\times 1000$.



PROPERTIES OF IONISED CELLS IN TISSUE DIFFERENTIATION AND ORGANISATION IN CHICK EMBRYOS

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ABSTRACT

The effect of ionisation on the structural organisation of chick embryos has been analysed. The radiosensitiveness of embryonic cells is in direct proportion to dosage. All initial effects of irradiation in blastoderm upto 600r are regulated and normal ontogenesis follows thereafter. The organisation of the embryo is suppressed at 2000r—cells become incapable of building up any pattern. Secondary neural axis may result at lower dosages, between 100r and 200r. Secondary inductions are suppressed at about 1000r. The various actions of radiation in the blastoderm have been discussed in terms of activity of gastrulating cells.

INTRODUCTION

The biological effects of radiation have been shown in a variety of tissues and the outstanding characteristic of radio-biological responses of the living system is their diversity. The main line of evidence of ionisation experiments as pointed out by Hevesy (1945), shows that X-rays mostly interfere with the mitotic-complex and to a minor extent with respiration and glycolysis of cells. The prime effect discovered is the suppression of cell division and there is a large amount of data on the radiation effects on chromosomes which have been admirably reviewed by Lea (1946) and Muller (1954). However, the effect of radiation on embryonic development needs intensive study; because in embryonic development there is not only active cell-division but also a series of substance-changes as pointed out by Needham (1942), Brachet (1950) and Waddington (1952). It is likely that irradiation interferes with cell division and respiration of cells. Borgonie and Tribondeau (1906) have long ago formulated the idea that active proliferating cells are more sensitive to radiation, and radiosensitivity varies inversely with degree of differentiation.

Attempts, so far made, to study the effects of irradiation on chick embryos, have resulted in the production of teratological abnormalities and have yielded little information on the mechanism of organisation. Wolff (1934) made an extensive survey of developmental irregularities obtainable after X-ray irradiation. However, his results have little importance, as pointed out by Waddington (1952), in the understanding of early developmental mechanism in chick embryos.

The effects of irradiation on mammalian development have been studied by Russell (1954) and Wilson (1954). Their observations deal mostly with later embryological abnormalities. Rugh (1954) has considered radio-susceptibility of amphibian embryos and concluded that radio-sensitivity of the embryo varies from stage to stage. According to him, radiation response of the gastrula is intermediate between the highly radio sensitive fertilised egg and blastula and the comparatively radio resistant ovarian egg, and the later neurula and tad-pole stages.

As a means of gaining understanding of the factors affecting the organisation in the chick embryos under the influence of radiation, this study was undertaken.

MATERIAL AND METHODS

At the crucial stage of gastrulation, the unincubated blastoderm *in ovo* was irradiated. Window holes were made in the eggs to determine the stage of blastoderm and after irradiation they were sealed again by paraffin. The source of radiation was a G.E.C. Million Volt X-ray Generator housed at the Chittaranjan Cancer Hospital, Calcutta.

The minimum dose employed was 50r and the maximum, 2000r, both inclusive of 14 per cent of back scattering. The intervening dosages were 75r, 100r, 125r, 200r, 300r, 400r, 500r, 600r, 700r, 800r, 900r, 1000r, 1100r, 1200r, and 1500r. After irradiation at the desired dose, the eggs were put back into the incubator almost immediately. Total number of experiments performed was 200. After each irradiation, blastoderms were fixed in Bouin's fluid in two series,—one set after 24 hours and another set after 48 hours. Serial sections of embryos were cut at 10μ and stained in dilute Delafield's hematoxylin.

EXPERIMENTAL RESULTS

Nature of Sensitivity of Blastoderm to Radiation Dose:

Visible effects of radiation are noticed only when the dose is over 600r. The nature of radio-sensitivity of the blastoderm below 600r is interesting from many points of view. Radiation below this dose may show initially a tendency of loose attachment of the developing cells but after 48 hours of incubation, this looseness is no longer visible in the embryo which passes on to normal gastrulation (Fig. 4.).

Normal development does not occur if the dose-limit is increased to 900r and above. Whatever may be the initial effect of looseness of cells produced in these blastoderms, they, after 48 hours of incubation, bear signs of radiation damage. At this dosage, gastrulation in these embryos is not normal, the neural tube is not properly thickened and the mesodermal elements do not separate well. The notochord is not present. Further increase of dosage affects the pattern of embryonic organisation. Often, failure of secondary induction is seen in blastoderms irradiated at 1000r (Pl. III, Fig. 3). The blastoderms, irradiated at 1100r, show these defects in a much more pronounced manner (Fig. 5). The cells show extensive cytolysis, the nuclei are enlarged and cellular attachments are loose. If these embryos are examined after 48 hours of incubation, they bear many important evidences of radiation changes. The ectoderm cells are not thickened into a well differentiated neural structure and do not form any tube. The mesodermal cells are significantly fewer. The somite-notochord differentiation is imperfect. A cluster of mesodermal cells occupies the middle region of the embryo.

Normal development is further inhibited when blastoderms are irradiated at 1500r. The embryonic axis shows a thin thickening of the epiblast cells whose true neural nature is doubtful (Pl. III, Fig. 1). Below the thickened area of the outer layer, some undifferentiated yolk endoderm cells are visible which do not form any distinct structure. No differentiation of mesodermal cells is seen. Even the thickening of the outer epiblast layer does not occur in a blastoderm which has received radiation at 2000r. The blastoderm is a sheet of undifferentiated cells without any structural organisation. The differentiation of epiblast and hypoblast in the blastoderm is not seen (Pl. III, Fig. 2).

In a few cases double neural formation has been noticed in the irradiated blastoderms (13 out of 200). Double axis formation occurs most frequently between dose levels 100r and 200r. The secondary neural axis is apparently a result of radiation. A representative case of double neural formation is shown in Pl. III, Fig. 6. A blastoderm, irradiated at 200r and observed after 48 hours of incubation, presents a double neural tube in the embryo. The secondary neural tube,

very small and circular in shape in cross-section, lies on the extreme left side of embryo. The extra neural tube is not accompanied by any somital or notochordal tissue. The neurocoele is distinct. The primary axis is characterised by the absence of uniform neural areas though the neurocoele can be marked out. Some chorda cells are also identifiable below the neural mass.

Double neural formation seldom occurs at a higher dose. One case observed at 1500r merits recording. Duplication is seen at the anteriormost end of the embryo; two neural tubes run parallel for a short distance, after which the original tube runs backward without being accompanied by the secondary neural tube.

Regulative Properties of the Irradiated Blastoderm:

The phenomenon of regulation shown by the cells of the irradiated blastoderm is remarkable. All effects of irradiation under 600r become untraceable in course of incubation and such blastoderms differentiate into normal embryos except for double neural tubes described above. However, the capacity of cellular regulation works within a physiological limit and if the blastoderm is irradiated at high dosage, the recovery of cells is inhibited. The cells after irradiation become loose and show signs of sublethal cytolysis—each having an enlarged nucleus and a thin layer of cytoplasm. This condition seems temporary because cells are capable of rearranging their developmental fate so as to pass on to normal trends of differentiation. The power of regulation of the embryonic cells is operative till 600r. Pl. III, Fig. 7 depicts a case of a perfect rearrangement of cells after 48 hours of irradiation at 100r. Normal tissue separation and structural organisation of cells occur in the regulated embryos. A similar case of perfect regulation after 48 hours has been shown in Fig. 4. The blastoderm was previously irradiated at 300r.

Loss of Organising Power of Cells Under High Frequency Radiation:

The regulative power of the blastoderm cells becomes progressively lost with the increase of dosages of X-rays. It has already been pointed out that radiation defects make their appearance at 600r after which the capacity for compensating the defects becomes ineffective. The effect of 1500r on the blastoderm needs mention. The regulative mechanism of the cells becomes inactive. The cells of the blastoderm have not been able to rearrange and organise themselves into any distinct pattern. The mechanism of tissue-separation has become inactive (Pl. III, Fig. 1). Neural differentiation and endoderm formation are not seen,—the blastoderm has fallen short of the normal structures. This can be taken as evidence of incapacity of the blastoderm to differentiate normally any more. Complete failure of differentiation is noticed in blastoderms irradiated at 2000r. They show enlargement without any organisation (Pl. III, Fig. 2). There is no axis and the area pellucida also cannot be distinguished. This is the final picture of incapacity of the blastoderm cells resulting from high dosages of X-rays.

DISCUSSION

From the foregoing observations it seems clear that the effects of irradiation of chick embryos bear a relation with the dosage. Higher doses invariably interfere with the process of embryogenesis and bring forth developmental abnormality in irradiated chick embryos. In another communication, we have shown that DNA, RNA and alkaline phosphatase are progressively denatured with radiation (Mookerjee and Bose, unpublished). During irradiation, water contents available to the cell become easily oxidised which is mainly taking place by the powerful oxidizing radicals OH and O_2H and less effectively by H_2O_2 (Guzman Barron, 1954). However, when the question of embryonic regulation comes for discussion, the

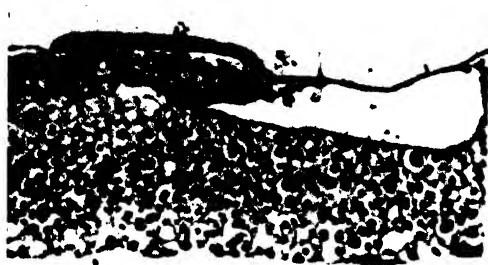
situation is not easy to explain. Whatever the exact biochemical processes involved in radiation damage, ionised cells, under the dose limit of 600r, can compensate all the initial effects and are capable of becoming normal embryos. It is clear that there is some mechanism at work after irradiation which restores normalcy. Higher frequencies of radiation injure this mechanism which becomes completely lost if the dosages of radiation are over 1000r. Burnst (1952) has also considered the possibility of recovery in ionised cells, though Rugh (1954) is in doubt about the operation of such a phenomenon. However, so far as this study is concerned, it can be advanced that within certain dose-levels, ionised cells show an unmistakable tendency of recovery.

In this connection, some of the post-irradiation effects observed by Rugh (1954) in amphibian embryos may be worth discussing. He describes failure of head differentiation at 360r and exo-gastrulation at 720r upon the blastula stage. He has also seen nonappearance of the neural tube when irradiation is done in the neurula stage. The manner in which the neural tissues are left undifferentiated in the experiments of Rugh seems to have a close parallelism with our case. However, it must be pointed out that the stage of development at which irradiation was done is different in the two cases. Rugh irradiated embryos after the organiser action was over and in our experiments it was done during organiser action.

There is some evidence that irradiation induces the production of double neural axis in chick embryos. The secondary inductions generally occur between 100r and 200r, they are only rarely produced at higher dosages. It is possible that irradiation produces a local condition of mild cytolysis which becomes the source of a secondary neuralisation. Mookerjee (1953) has advanced the notion about 'types' of cytolysis favouring particular trends of differentiation. It is not unlikely that ionisation is capable of producing a precondition for a secondary induction in an irradiated blastoderm. The phenomenon of morphogenetic movements during gastrulation should not be lost sight of in any discussion of organisation. On irradiation, cell-movements (Pasteels, 1945), may be affected, giving cause for subsequent abnormal differentiation. Waddington (1952) stresses the importance of this in discussing the problems of epigenetics of birds. In irradiated embryos, such morphogenetic movements are likely to be disturbed.

EXPLANATION OF PLATE

- Fig. 1. T. S. irradiated embryo (1500r) after 48 hours incubation. Note thickened plate-like ectoderm and a large space below the epiblast.
- Fig. 2. T. S. irradiated embryo (2000r) after 48 hours incubation. Note complete absence of any structural differentiation; blastoderm remains as a sheet of undifferentiated cells.
- Fig. 3. T. S. irradiated embryo (1000r) after 48 hours incubation. Note absence of lens placode in front of the cup.
- Fig. 4. T. S. irradiated embryo (300r) after 48 hours incubation. Note normal differentiation of nerve tube, notochord and somites.
- Fig. 5. T. S. irradiated embryo (1100r) after 48 hours incubation. Note thickening of the ectoderm and some mesodermal cells; no notochord present.
- Fig. 6. T. S. irradiated embryo (200r) after 48 hours incubation. Note presence of two neural formations.
- Fig. 7. T. S. irradiated embryo (100r) after 48 hours incubation. Note regular neural formation, notochord and heart.



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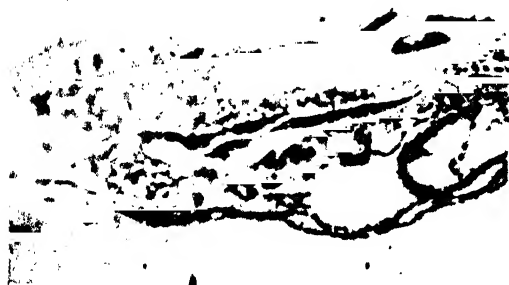
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Cell-division, which is also important in morphogenesis, seems to be affected in embryos on irradiation. As long as the power of recovery in the cells remains, it is probable that cell-division is ultimately completed. However, when irradiation incapacitates the inherent tendency of regulation, it leads to failure of production of normal types of cells. Such embryos lack all the cellular elements after gastrulation. This amounts to failure of individuation of the embryo. The effects of ionisation, especially at higher dosages, are evidently far-reaching and produce many embryological inhibitions and retardations of cells. The blastoderm remains as a sheet of cells without any sign of structural differentiation.

ACKNOWLEDGEMENT

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EFFECTS OF GAMMA RADIATION ON NITROGENOUS END PRODUCTS IN URINE OF ALBINO RATS

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ABSTRACT

The effect of whole-body radiation on a few groups of albino rats by different doses (1200r, 600r, and 300r) of gamma radiation from a Co^{60} source has been studied, mainly in the excretion pattern of the nitrogenous end-products in the urine, with the hope of finding out any indication of derangement in the pathway of protein metabolism in the body. In the 600r and 300r groups free ammonia, urea, uric acid and creatinine increased following irradiation within 24 hours and came down to normal values within 2nd and 3rd week. In the 1200r group a second rise occurred in the excretion of free ammonia and urea following the first rise. As for the amino acids no significant change was detected except in the 1200r group where the excretion of glutamic acid, glycine, α -alanine, valine and phenylalanine was increased. No increase of taurine was observed but the excretion of total sulphur was significantly increased. The large increases in the urinary output of free ammonia and total sulphur are the most important observations of the present work and it will be of interest to direct further investigations as to the causes of such increase.

INTRODUCTION

Effects of high energy radiation on biological systems are complicated and extensive all over the tissues of the body. Following total body exposure to ionising radiations, various degrees of damages are known to occur in almost all the tissues in animal systems and even may result in death. Analysis of biological fluids, particularly of urine, reveals quantitative and qualitative changes in the excretory products as a result of exposure to radiation; these data may be of significance in studying the changes induced in the various organs and tissues due to radiation damage.

Although the exact mechanism of radiation damage is as yet obscure, the response of the living organism as a whole is dependent upon the dosage employed. If animals are exposed to severe dosage of radiation, apart from immediate effects of shock and tissue destruction progressive delayed effects continue to occur resulting in death. These delayed effects are particularly noted in the case of protein metabolism: tissue break-down goes unchecked without any net synthesis.

In the present investigation, urinary excretory pattern in albino rats has been noted following exposure to moderate, large and excessive dosages of gamma radiation, with a view to studying the departures from normal pattern and also to find out the peculiarities, if any, of the irreversible changes occurring within the organism, when exposed to lethal dose of radiation.

As the total nitrogen excretion in urine was noted to be increased in an earlier experiment by the authors (Das, Dutt and Mukherjee 1957), the major end products of protein breakdown have been recorded in this work in terms of urea and NH_3 . Among the different products of purines and other non-protein nitrogenous constituents studied, emphasis has been laid only to uric acid and creatinine excretion. Apart from these, urinary amino acids have been estimated, as excessive increase of a large number of amino acids in urine was reported to occur following accidental exposure to ionising radiation (Jasterlik and Marinelli, 1955). Such rise was also

known to occur in animals after exposure to x-irradiation (Kay *et al.*, 1954). Apart from these taurine and total sulphates have been estimated. Kay *et al.* studied taurine excretion following x-irradiation and noted significant increase (Kay *et al.*, 1957). Barron *et al.* showed that sulphhydryl containing enzymes are particularly inactivated by X-rays and it is due to the oxidation of the -SH linkages by the highly oxidising free radicals produced by the radiolysis of water, present in biological systems. (Barron *et al.*, 1949). The major end product of these enzymes is taurine. From the same consideration, changes in sulphur excretion should also appear significant.

In order to remove extraneous factors as far as possible and to ensure uniformity of results, animals were given ample time to acclimatize themselves with the experimental conditions. Regular analysis for a considerable period was done prior to exposure to radiation: same procedure was maintained following irradiation until the pattern came back more or less to normal or resulted in death. During the experimental period no unnatural conditions, such as fasting, salt restriction etc., were permitted.

EXPERIMENTAL

30 rats—all females weighing between 120 gm. and 180 gm. were divided into 5 groups of six rats in each group. The rats of each group were kept in two metabolism cages—3 in each cage. Urine samples from each cage were collected under a layer of toluene every 24 hours. Urine samples of each group were mixed and filtered into a 100 ml. volumetric flask and diluted upto the mark. All the analysis were done with this dilute urine.

Urinary creatinine was estimated according to Saffir's modification of Folin's method (Hawk *et al.*, 1954). The colour developed was measured photometrically at 520 *mμ*. Uric acid was measured according to the method of Christman and Ravitch (Christman *et al.*, 1932). The colour produced was measured photometrically at 700 *mμ*.

The urea content was estimated by the modified urease method as outlined by Van Slyke and Cullen (Hawk *et al.*, 1954). Urinary free ammonia was estimated by a modification of the method suggested by Ma and Zuazaga (1942) for the micro-kjeldahl determination of nitrogen. 1 ml. of the urine sample was taken in the distilling flask of a microkjeldahl apparatus and steam was passed through it without the addition of any alkali. The distillate was absorbed in 2 percent boric acid solution and titrated with standard HCl solution using a mixed indicator consisting of 1 part of methyl red and 5 parts of bromo cresol green.

The inorganic sulphate content of the urine was measured by precipitating the sulphate as benzidine sulphate by shaking 2.5 ml. of the diluted urine with benzidine solution (Hawk *et al.*, 1954). The precipitated benzidine sulphate was made free from acid by repeated washing and centrifugation with 90 percent acetone saturated with benzidine sulphate. The washed precipitate was then dissolved in boiling water and was titrated against standard NaOH solution. In case of total sulphur all the sulphur content in the urine which is not present as sulphate is oxidised to sulphate by the method of Benedict (Hawk *et al.*, 1954), and the total sulphur was estimated as sulphate according to the method referred to in the case of inorganic sulphate.

The amino acids including taurine were separated and estimated by two dimensional paper chromatographic method using water-saturated-phenol and a mixture of *n*-butanol-acetic acid-water (4 : 1 : 1) as the two solvents. The spots were developed, dried and eluted and the ninhydrin colour densities were photometrically measured according to Kay *et al.* (1956).

Prior to chromatography the urine samples were desalted, as otherwise, the salt present in the urine gave rise to serious trailing. The desalting was carried

out by passing 10 ml. of the slightly acidified urine sample through a column 15 cm. in height and 1.5 cm. in diameter of "IR-120". The resin bed was then washed with 100 ml. of distilled water (CO_2 -free) at the rate of 6 to 8 drops per minute. The effluent contained all the taurine and the acidic peptides. The amino acids were all retained by the resin which were subsequently eluted by means of 50 ml. 2N ammonia followed by 50 ml. of distilled water for another fraction. Both these fractions, one containing the whole of the taurine and the other the rest of the amino acids were evaporated to dryness and the residues were treated with 1 ml. of distilled water each to extract all the amino acids. The extracts were centrifuged and 0.1 ml. of the supernatant from each fraction was applied to paper for chromatography. Two different chromatograms were prepared for the taurine fraction and the amino acids fraction.

After taking normal readings for seven days 3 groups of the rats were exposed to gamma radiation from an about 20 curie Co^{60} source. The doses given to the three groups were about 1200r, 600r and 300r respectively. The rats were put individually into small wire cages to restrict their movement. The cages were fixed on a wooden platform along the circumference of a circle of proper radius. The source was then placed at the centre of the circle by means of a remote control mechanism. Two groups of rats were not irradiated and were kept as control.

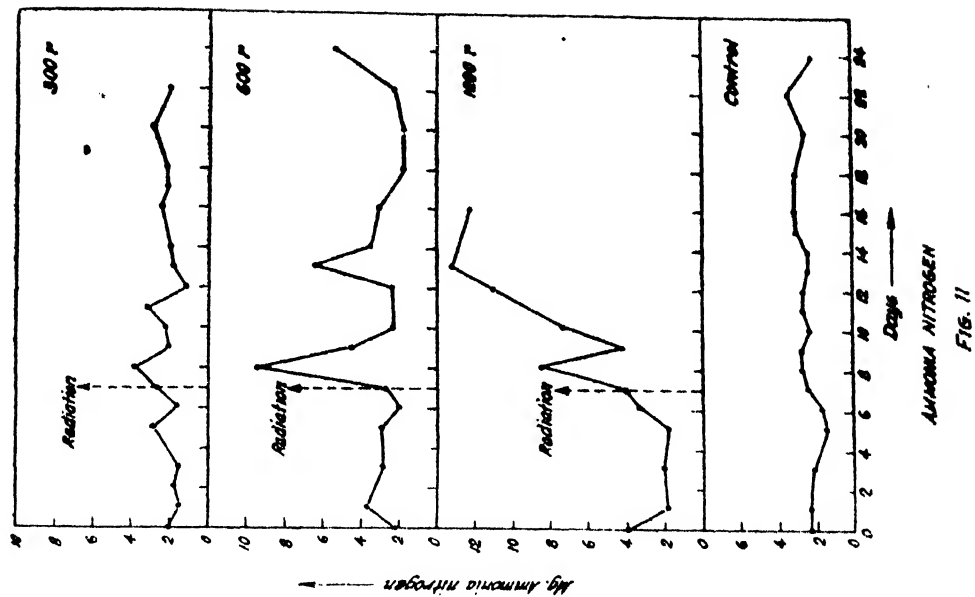
RESULTS AND DISCUSSION

The normal excretory pattern of the rats, as studied in groups of 6 animals, showed similar characteristics. The 24-hour sample of urine, however, showed some degrees of fluctuations of the different constituents from day to day and therefore a mean value was accepted as the standard one. The amino acid pattern of all the groups showed the presence of identical spots, some of them appearing regularly such as glutamic acid, glycine, alanine, valine and phenylalanine. Apart from these, presence of aspartic acid, lysine, tyrosine, methionine, leucine and hydroxyproline were identified; but they appeared either irregularly or in very small amount and as such they were neglected.

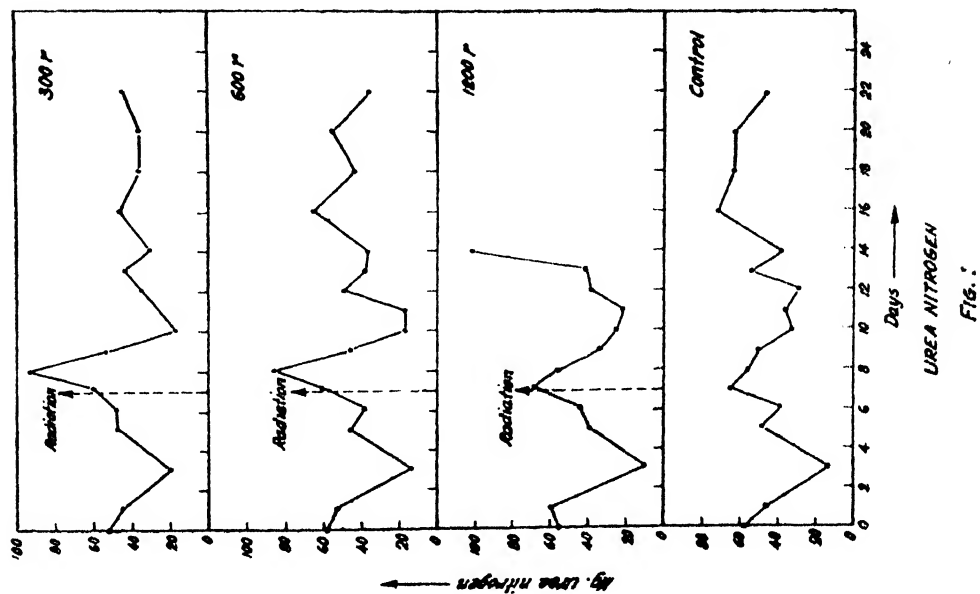
The animals exposed to 300r behaved almost normally. Those exposed to 600r showed epilation, lassitude, anorexia, and emaciation; the condition improved at the end of 2nd week. Intraocular tension and proptosis developed in all the animals exposed to 1200r and 600r. All the animals exposed to 1200r died within the first post-irradiation week. In this group, animals became extremely feeble, averse to food and refractile to external stimuli; epilation was extensive, convulsions also occurred. Morbid anatomy following post-mortem examination showed massive haemorrhage in brain, stomach and small intestine, acute dilatation of stomach and small intestine was also noticed. Blood examination 24 hours after irradiation showed leucopenia and erythropenia in all the groups after radiation; however, the fall of the leucocyte and erythrocyte counts was proportional to dosage.

Urinary analysis showed that urea, free NH_3 , uric acid and creatinine increase following irradiation (Figs. I, II, III and IV). This increase took place in the immediate 24 hours following exposure and came down on the 2nd or 3rd day. In the cases of 300r and 600r groups a second increase was observed which persisted during the first post-irradiation week, but gradually came down to normal during the second or third week. In normal animals the amount of free ammonia was persistently very small, but a peculiar phenomenon was noted in irradiated animals, when considerable amount of free NH_3 occurred in urine and the amount was roughly proportional to dosage.

The 1200r group differed considerably from the other two groups. In this group following a transient rise in the 24 hours immediately following irradiation, urea and free ammonia came down as was noted in other groups; the fall was more



AMMONIA NITROGEN
FIG. 11



UREA NITROGEN
FIG. 12

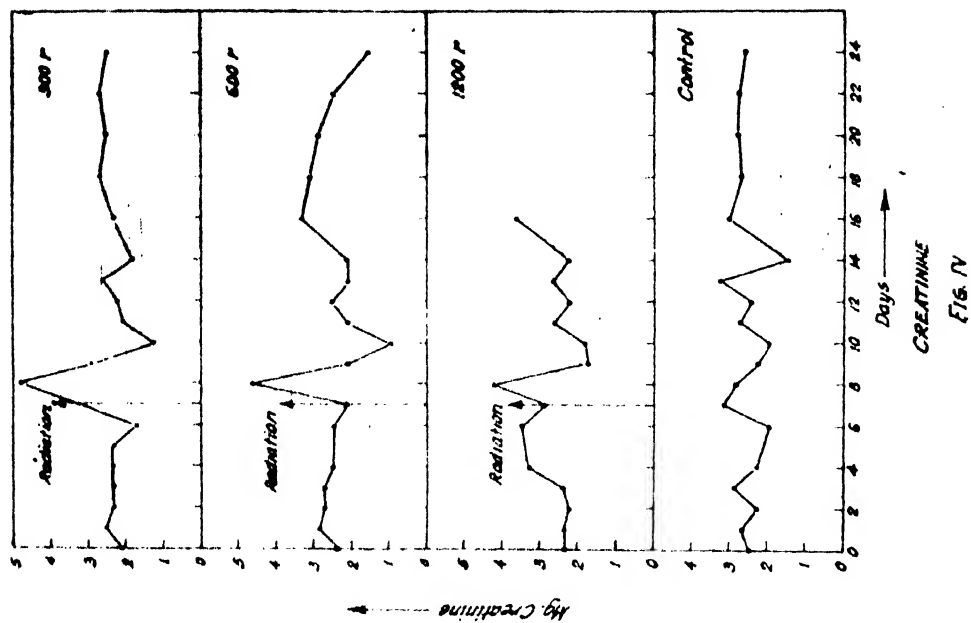


FIG. IV

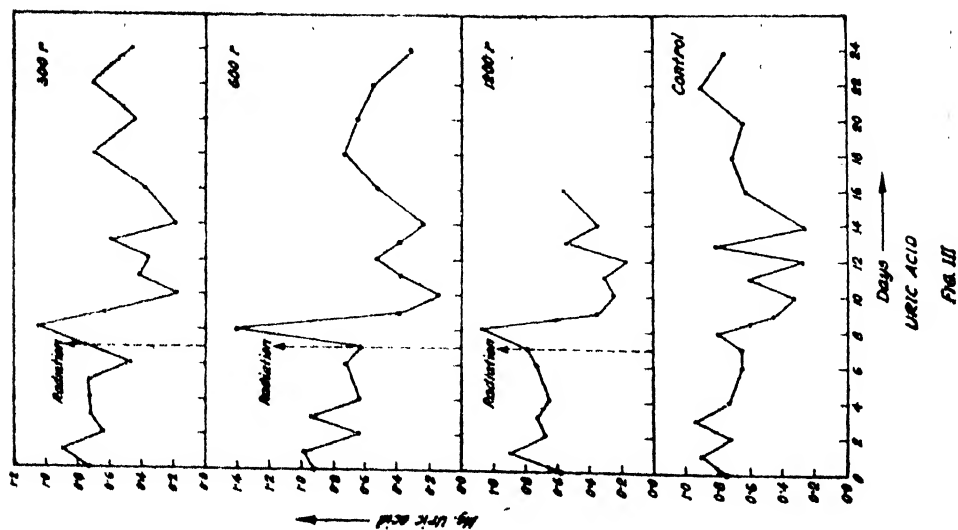


FIG. III

marked in this case. The uric acid level after coming down, persisted to show low values, as compared to normal. However, from the 3rd/4th day urea and free NH_3 excretion gradually continued to rise till death supervened.

The two peaks in these excretory curves conform to the immediate and delayed changes in protein metabolism resulting in tissue destruction; however the significance of free ammonia is obscure. It may be that the ammonia pool of the system, notably glutamine, is depleted giving rise to the free NH_3 in urine. It would be interesting to study the concentration of NH_3 in different tissues following irradiation and also to correlate between the amino acid nitrogen levels in blood of irradiated animals with the NH_3 -urea excretion in urine. Such experiments are now being carried on in this laboratory. In any case, it seems that urinary ammonia excreted comes from a highly labile compound which is easily decomposed by steam.

Another peculiar feature noted was the excretory pattern of taurine in urine. There was no significant rise in taurine level in the urine of the irradiated rats, although inorganic sulphates increased and the increase was roughly proportional to dosage (Figs. V and VII). Although evidences suggest that SH-containing molecules are converted to taurine in liver and a few other organs, such as kidney and spleen, the excretion may not invariably take place as such. It is known that taurine is present widely in animal tissues and its chief metabolic product is inorganic sulphate in urine. In another series of experiments with X-rays, taurine increase was noted by the present group of workers, which was not very high and also did not persist for a significant period of time (Das, Dutta and Mukherjee, *to be published*). It appears that a more reliable data should be the excretion of inorganic sulphate and total sulphur indicating the level of SH-containing molecules and other sulphur containing molecules respectively. In the present work such data are consistent with the expected scheme of events. In moderate and severe exposure, these sulphates are increased in urine but gradually come down and attain normal levels. While in the group exposed to 1200r, these excretion curves swing upwards till death (Figs. VI).

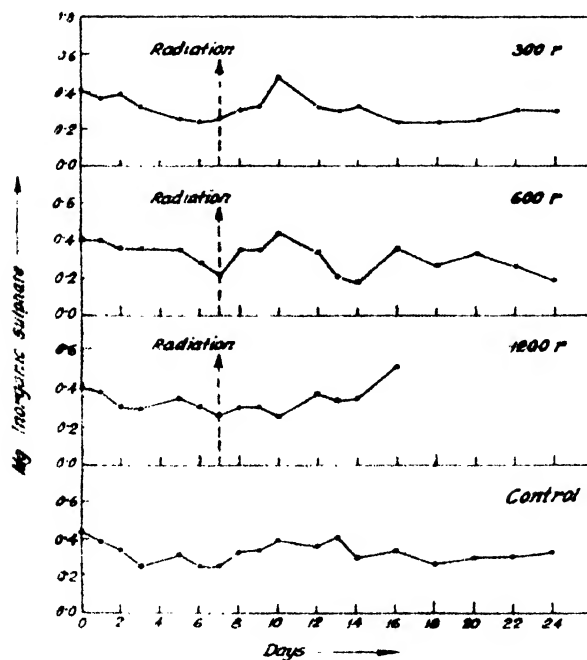
Regarding other amino acids, persistently excreted by the irradiated animals, only the 1200r group shows significant increase of glutamic acid, glycine, α -alanine, valine and phenylalanine (Figs. VIII, IX, X, XI and XII).

A comparative study of the excretory pattern of the groups exposed to different degrees of radiation damage shows certain common features. There is an initial rise in the major nitrogenous excretory products in urine which take place within the first 24 hours after radiation. This is presumably due to the immediate tissue destruction. This rise comes down within normal limits within 2/3 days. But soon after a more gradual increase of the nitrogenous excretory products takes place which gradually tend to come down within the 8th or 10th day. But where extremely severe dosages are employed, this secondary rise has no tendency to come down and death occurs at the peak of the excretion curve. Further work regarding the detailed mechanism, responsible for the changes noted, is being conducted at present in this laboratory.

ACKNOWLEDGEMENT

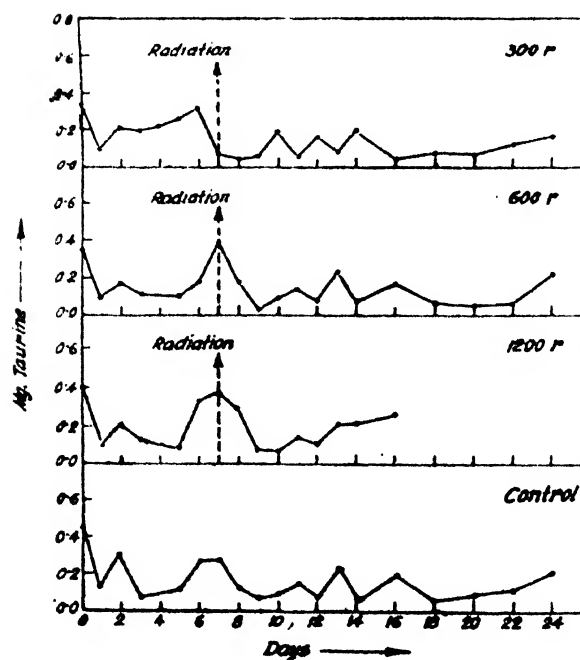
Thanks are due to Dr. D. M. Bose, F.N.I., Director, Bose Institute, for his kind and valuable guidance during the present work. The authors are also grateful to Dr. P. K. Bose, F.N.I., Head of the Department of Chemistry, Bose Institute, for his interest and encouragement.

Sri A. M. Ghose of the Bose Institute and Dr. J. J. Ghosh of the University College of Science, Calcutta rendered invaluable assistance during the experiments; Dr. S. P. Sen and Dr. D. P. Burma were very kind and generous with their valuable advice.



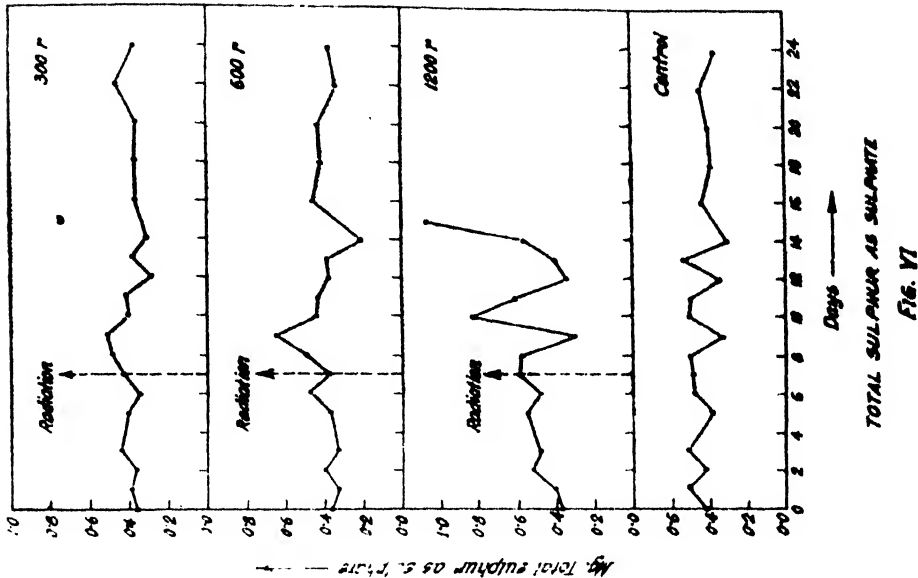
INORGANIC SULPHATE

FIG. V

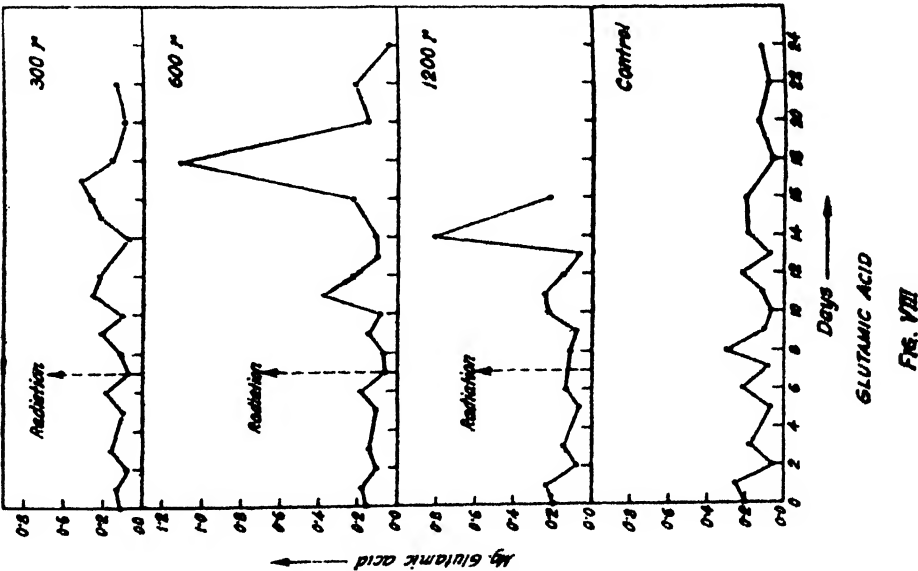


TAURINE

FIG. VI



TOTAL SULPHUR AS SULFATE
FIG. VI



GLUTAMIC ACID
FIG. VII

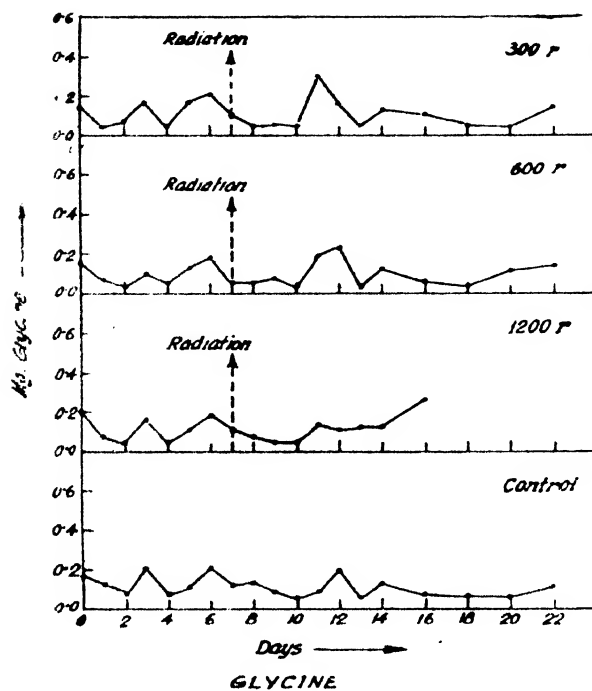


FIG IX

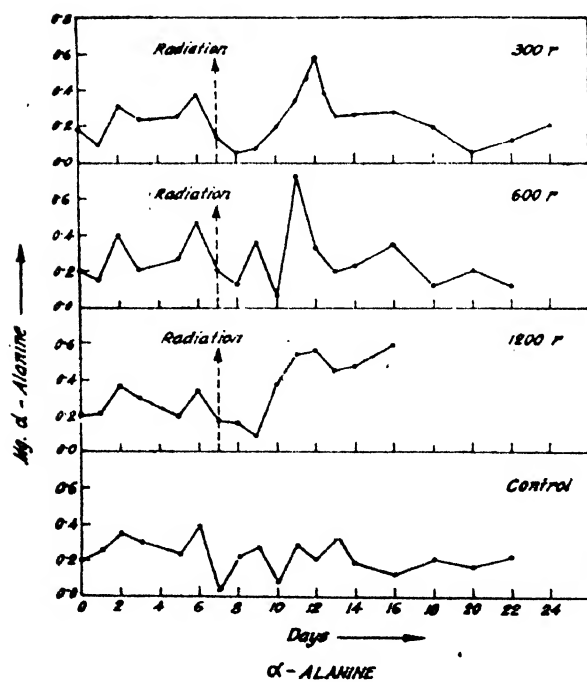


FIG. X

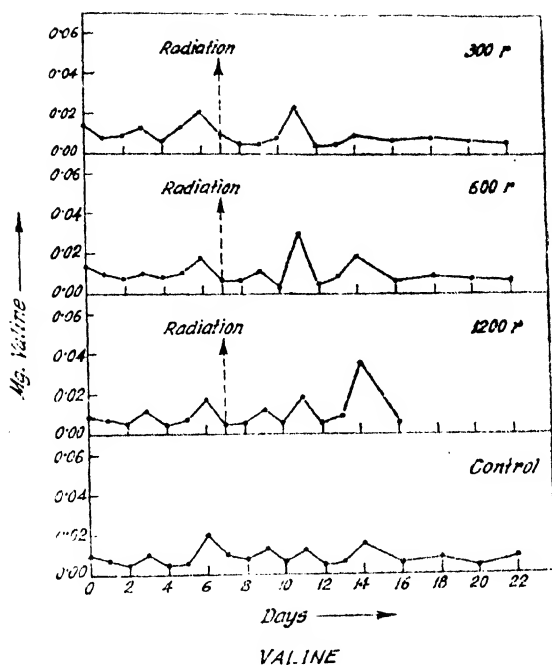


FIG. XI

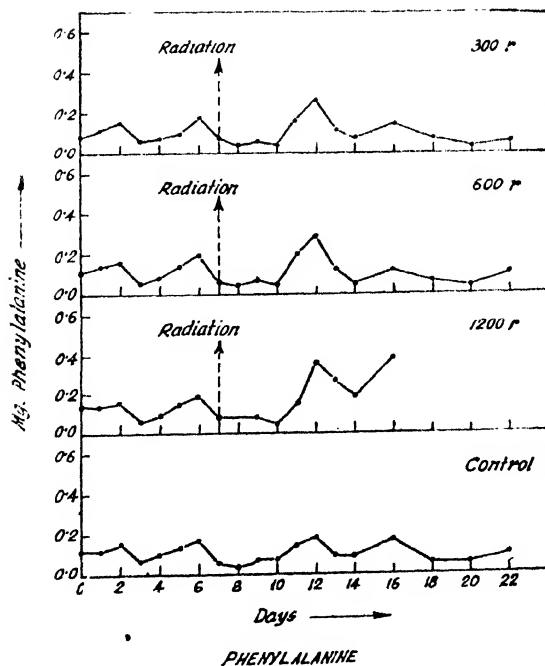


FIG. XII

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TWO SPECIES OF ENCYRTIDAE PARASITIC IN THE PINK MEALYBUG OF SUGARCANE IN INDIA—[HYMENOPTERA]

by HAROLD COMPERE,¹ B. R. SUBBA RAO and R. B. KAUR²

(Communicated by E. S. Narayanan, F.N.I.)

(Received August 8; read October 2, 1959)

ABSTRACT

In the winter of 1957, some parasites of the sugarcane mealy-bug *Saccharicoccus sacchari* (Ckll) were reared in the Entomology laboratory of the Indian Agricultural Research Institute, Delhi. Among these were two species of Encyrtidae of unusual interest owing to their structural peculiarities and have thus been described in detail. The genus *Scelioencyrtus* Girault was synonymized with *Xanthoencyrtus* Ashmead by Timberlake (1920) on insufficient grounds. *Scelioencyrtus* Girault has now been reinstated because of the peculiar structure found in the male antennae. A key to the species of *Scelioencyrtus* females has also been prepared. *Astymachus japonicus* Howard has been recorded from India for the first time and has been redescribed.

FOREWORD

by HAROLD COMPERE

Among some chalcidoid parasites received from India were two species of Encyrtidae reared from the pink mealybug of sugarcane, *Saccharicoccus sacchari* (Ckll.), collected near Delhi by Kaur and forwarded by Subba Rao. These encyrtids were of unusual interest owing to their structural peculiarities and what appears to be a correlation between structure and habit. Both species appeared to be specialized for crawling between the sheaths and culms of graminaceous plants in searching for hosts, but poorly developed for jumping and flying. The dried specimens of both species were badly shrunk, distorted, and paper thin—the heads flattened fronto—occipitally, and the bodies flattened dorsoventrally; the coxae and femora of the hind legs slightly swollen, the middle legs weak with small tibial spurs; the setae on the head and body small and scarcely perceptible. One species was extremely elongate, with a long, strongly exerted ovipositor, and the other less elongate, with an exceedingly small, concealed ovipositor. I advised the sender that the species with the long ovipositor was an *Astymachus* and referred the other species to *Scelioencyrtus*, and stated that before the species could be determined a more comprehensive study should be made of the species in the genera *Xanthoencyrtus*, *Mirastymachus*, and *Pholidoceras* and suggested that he do this. Shortly afterwards my interest in the species was reawakened by Dr. Ishmail Famy, a visitor to Riverside from the Sudan. He reported that the mealybug, *Saccharicoccus sacchari*, was a very serious pest of sugarcane in Egypt and it was feared it would spread to the Sudan and might ruin the sugarcane industry there. He expressed a hope that the mealybug might be controlled in Egypt by the introduction of its parasites before it spread to the Sudan. I restudied the two species of Encyrtidae reared from this mealybug in India. The specimens of *Astymachus* proved to be indistinguishable from specimens of

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A. japonicus Howard. The sample of *japonicus* from Japan was a gift from Professor Keizo Yasumatsu, and consisted of three females, reared from *Aclerda tokionis*, collected at Fukuoka, Kyushu, June 15, 1956, by Y. Murakami. In the meantime, Subba Rao and Kaur prepared and sent for review an article on some new species of Encyrtidae from India, which included the two species under consideration here, and I was named as the senior author. This article is a compromise and concerns only the two species in which I had a special interest. In the case of the new species, *mymaricoides*, much that is stated in connection with its generic status is a matter of opinion only, and I am solely responsible for these opinions.

Astymachus and *Scelioencyrtus* have a number of structural peculiarities that may prove instructive to expert anatomists in tracing the evolution of the thorax in the Encyrtidae.

SCELIOENCYRTUS Girault

Scelioencyrtus. Girault (1915), *Mém. Q. Mus.*, 4, 161.

Remarks : There is a possibility that Timberlake, (1920) synonymized *Scelioencyrtus* with *Xanthoencyrtus* Ashmead on insufficient evidence. The species *mymaricoides* described below is more closely related to *Scelioencyrtus nigriclavus* Girault, the genotype, than any described species, but it differs too greatly from *Xanthoencyrtus sensu stricto* to be placed in this genus. *S. nigriclavus* was described as having longer marginal fringes than usual—a fifth the greatest wing width. In *mymaricoides* n. sp., the fringes of the front wings are about one-half the greatest wing width, and extend from near the stigmal vein on the anterior margin to a point opposite the stigmal vein on the posterior margin, and the fringes of the hind wings are longer than the greatest wing width. In *Xanthoencyrtus* the marginal fringes are short, and in the males the sixth segment of the funicle is normal in size and shape with a row of erect, club-shaped setae, or sensory organs. In *mymaricoides* the sixth segment of the funicle is enlarged in the males, one side concave with a group of crowded, circular, sensory pits, the opposite side convex, with the distal end flattened and slightly expanded. The final decision as to whether a mistake was made in synonymizing *Scelioencyrtus* will probably depend largely on the characters to be found in the male antennae of the species described by Girault under *Scelioencyrtus*, particularly *nigriclavus*. Girault described this and two other species from one female each, collected in Australia. If no mistake was made by Timberlake in synonymizing *Scelioencyrtus*, it may be desirable to erect a new genus for *mymaricoides*.

Major diagnostic characters of generic value are given in the description following the minor characters, in case the genus *Scelioencyrtus* is not reinstated and it is desired to erect a new genus for the reception of *mymaricoides*.

Scelioencyrtus mymaricoides, n.sp.

Distinguishable from all described species of Encyrtidae by the extraordinarily long marginal fringes of the wings, and in addition by the unusual sixth segment of the funicle in the male sex.

Minor characters.—*Female*.—General colour yellow to yellowish white, or pallid, with extensive brown to blackish suffusions. Frontal aspect of head, the greater part of mesoscutum, scutellum and axillae, abdomen near the base and the tergum of the tenth [morphological] segment yellow. Ventral parts of thorax and abdomen, largely if not completely, yellowish white, or pallid, with concolourous legs except for the pretarsi which are black. Antennae dark brown or blackish with the scape and pedicel more or less suffused with pale yellow. Cheeks and lower part of face between antennal sockets brown, occipital aspect of head con-

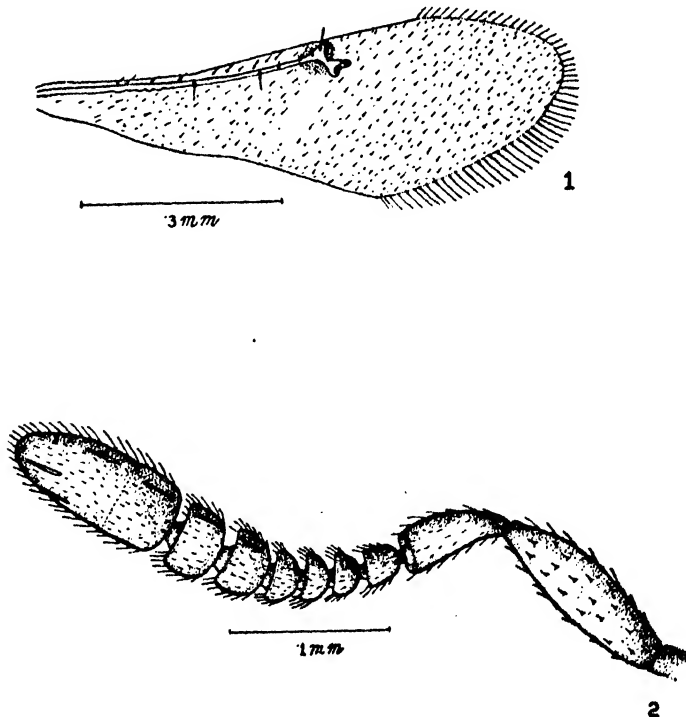
colourous with ventral thorax. Pronotum more or less, anterior margin of meso-scutum, lateral and posterior sides of scutellum, metanotum, more or less of mesopleura and basal wing processes brown to blackish. Propodeum pale brown, and somewhat whitish on abdomen at base grading to yellow and brown.

Frontal aspect of head, mesoscutum, axillae and scutellum very finely and closely reticulate producing a punctate effect, shining, exceedingly finely, sparsely pubescent, the pubescence scarcely visible at a magnification of 72x. Wings highly iridescent and with exceedingly fine, pale, scattered hairs that are scarcely visible in direct light, and which do not form a definitive speculum; the wing membrane appearing faintly wrinkled or roughened under high magnification.

Head wider than long, the eyes small, protuberant, about as long as the cheeks ocelli large, three times their own diameter apart, and in dried specimens almost in a transverse line across the vertex. Scape short and thick, not compressed below, slightly more than twice as long as wide; pedicel about one and one-half times as long as wide; first funicular segment slightly longer than wide, the succeeding four slightly wider than long, the sixth about as wide as long; club two-segmented, about as long as the preceding four funicular segments and slightly wider. One or two linear sensillae visible on each funicular segment when viewed in one focal plane; the hairs on the antennae moderately long, and scattered sparsely.

Front wing more than three times as long as wide, the margins almost parallel distad of the venation and almost evenly rounded apically. Submarginal vein long, weakly developed, terminating apically in an enlargement scarcely recognizable as the marginal and postmarginal veins; stigmal vein normal.

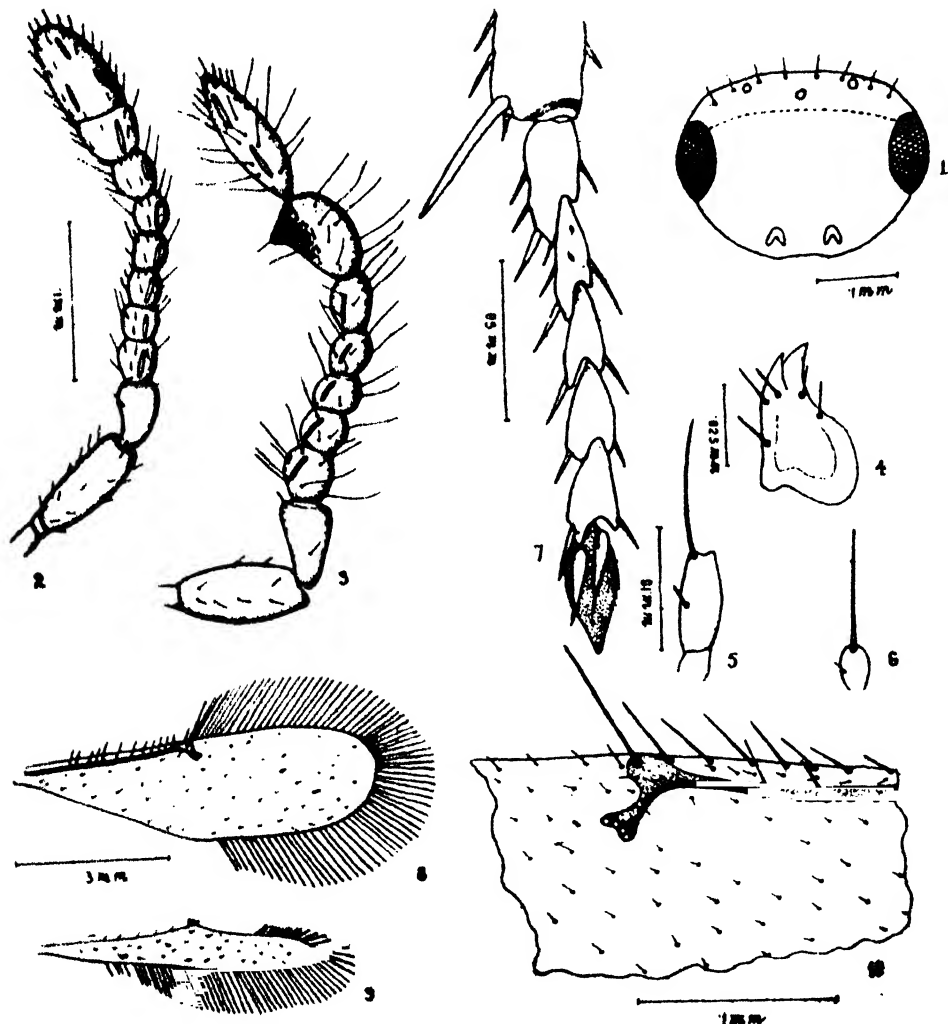
Length ranging between 1-3 mm.



TEXT-FIG. 1.

- Fig. 1. Fore wing of *Astymachus japonicus* Howard ♀
 2. Antenna of *A. japonicus* Howard ♀

Male.—Except for the antennae, the genitalia, and certain modifications of the sclerites of the abdomen, the males closely resemble the females. As to proportions and shape, there is a similarity between the two sexes with reference to the scape, pedicel and first five segments of the funicle, but with this the similarity between the antennae of the two sexes ends. In the males the sixth funicular segment is more than twice as large as the preceding segment, flattened and slightly widened apically, one side concave and the other convex, the concave side with crowded, circular sensory pits. Club small, solid, elongate, about three times as long as wide and uniformly and evenly tapered at both ends. Unlike the females, the flagellum is furnished with whorls of curved hairs.



TEXT-FIG. 2.

Scelioencyrtus mymaricoides, n. sp.

- Fig. 1. Head, front view ♀ 2. Antenna ♀ 3. Antenna ♂ 4. Mandible ♀
5. Maxillary palpus ♀ 6. Labial palpus ♀ 7. Mid tibial spur and tarsi ♀
8. Fore wing ♀ 9. Hind wing ♀ 10. Portion of the fore wing highly enlarged showing the vention ♀

Major characters.—Female.—Elongate, paper thin as noted. After drying specimens appear shrunken and distorted with the frontovertex and face directed dorsad and lying in the horizontal plane. Antennae inserted close to the oral margin; the head articulated to the cervical processes high above the center of the occiput. Mandibles with two teeth. Maxillary palpi two-segmented; labial palpi one-segmented. The apical dorsal sclerite (= tergum *x*) of the abdomen greatly enlarged and margined on either side by well-developed paratergites [= cut-off dorsal remnants of the tergum of segment IX]. Ovipositor exceedingly small and enclosed in the apex of the abdomen by sternum of the last apparent segment. Ovipositor sheaths [= styli] vestigial or absent. Inner and outer plates of ovipositor [= gonocoxites and gonotergites] scarcely more than twice as long as wide, if that long. Weakly if at all saltatorial, tibial spur of middle legs small and weak, about as long as the short basitarsus. Mesopleura only slightly enlarged and appearing in relatively simple form with a ridge [= pleural ridge ?] extending horizontally from the lateral coxal condyles to the posterior margin of the mesopleura. Mesosternum partially divided into three transverse subequal sections by two submedian sutures or ridges.

Except for the differences in the wings and in the sixth funicular segment of males as noted, *mymaricoides* does not appear fundamentally different structurally from several unidentified species of *Xanthoencyrtus* in the collection of the Citrus Experiment Station.

Described from 20 females and 4 males reared from *Saccharicoccus sacchari* (Kll.), collected by R. B. Kaur, Delhi, India, 1957. Three females and 3 males, all on slides and designated paratypes, in the "National Pusa Collection", Division of Entomology, Indian Agricultural Research Institute, New Delhi. Seventeen females and 1 male in the collection of the Citrus Experiment Station, Riverside, designated holotype, allotype and paratypes. The female holotype under one cover slip with 4 paratype females, and the male allotype under a second cover on the same slide, to be deposited in the U. S. National Museum, and paratypes in the British Museum of Natural History.

KEY TO THE SPECIES

Described under *Scelioencyrtus*, Females

- | | |
|---|------------------------------|
| 1. Front wings with the marginal fringes much shorter than one-half the greatest wing width..... | 2 |
| — Front wings with the marginal fringes one-half the greatest wing width..... | <i>mymaridoides</i> , n. sp. |
| 2. Front wings with the marginal fringes one-fifth the greatest-wing width..... | 3 |
| — Front wings with the marginal fringes very short, less than one-fifth the greatest wing width. First three funicular segments blackish, the distal three white..... | <i>tricolor</i> Girault |
| 3. Honey yellow; antennae dusky yellowish with the club jet black.... | <i>nigriclavus</i> Girault |
| | <i>keatsi</i> Girault |
| Marked with blackish; antennae jet black..... | |

ASTYMACHUS Howard

Astymachus, Howard, (1898), *Proc. U. S. nat. Mus.*, 21, 238; Girault, (1915), *J. N. Y. ent. Soc.*, 23, 167.

Remarks: This genus is not closely related to *Aphycus* with which it was compared by Howard, nor to *Xanthoencyrtus* with which it was compared by Girault. It is a distinctive genus not closely related to any other known to us.

It is suggestive of *Xanthoencyrtus* with regard to the flattened head and body, almost imperceptible hairs, two-segmented antennal club in the females and the row of short, erect, club-shaped setae on the sixth segment of the funicle in the males, but otherwise there is little similarity between the two. Although the tergum of the fourth segment is greatly enlarged and the ovipositor lies enclosed by the sterna in the apex of the abdomen, and not exposed as if in a groove, *Astymachus* belongs to the Mirini and *Xanthoencyrtus* and related genera to the Ectromatini.

Some Major Characters for Distinguishing Astymachus: Elongate, flattened ovipositor exerted one-fifth the length of abdomen. Head appearing flattened horizontally in dried specimens with the frontovertex and face dorsad in one plane, but after distension in acetic acid the head appears more vertical than horizontal with the vertex rounded and the face inclined ventrocaudad. Ocelli small, far apart and in an acute triangle. Antennae inserted near the middle of the face and the head articulated to the cervical processes slightly below the center of the occiput. Eyes small, shorter than the length of cheeks. Antennal club two segmented. Mandibles with three nearly equal teeth. Maxillary palpi four segmented; labial palpi three-segmented. Front wings moderately slender, almost three times as long as wide, almost evenly curved and rounded distad of the stigmal vein; marginal fringe moderately long, about one-fifth the greatest wing width; submarginal vein long and weak; the marginal vein scarcely distinguishable and narrowly separated from the costal margin; postmarginal vein rudimentary. Pronotum slightly longer than the mesoscutum; scutellum not evenly rounded but with the sides near the base almost straight. Mesophragma almost as long as the thorax and projecting into the abdomen almost one-third the length of the latter. Abdomen about twice as long as the thorax and slightly wider, widest near the cerci in the middle; tergum of segment X enlarged; paratergites absent. Ovipositor exceedingly long, the over-all length more than the length of the tibiae and tarsi of the hind legs together, and exerted almost one-fifth the length of the abdomen. Coxae and femora of hind legs distinctly swollen. Middle legs relatively weak, the tibial spur not as long as the basitarsus, the latter about one and one-half times as long as the following segments; front legs with relatively large curved femora. The lateral margins of the dorsal sclerites of the abdomen are infolded or thickened, and cleared, stained specimens the margins are visible in straight lines from near the basal to the apical end of the abdomen and produce an effect as if the abdomen walls were reinforced by longitudinal apodemes. In this genus, as represented by *A. japonicus*, the mesosternum is large, transverse and not divided by sutures or ridges, and the mesopleura relatively simple.

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PATTERN OF SUBSTANCE LOCALIZATION IN THE ALTERNATE EXISTENCE OF AMOEBA

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(Communicated by B. R. Seshachar, F.N.I.)

(Received September 2; read October 2, 1959)

ABSTRACT

A species of soil amoeba (*Acanthamoeba* sp.) has been studied cytochemically in trophic and cystic conditions. In trophic forms, localization of alkaline phosphatase is seen in the nucleus and in the cytoplasm. The basophilia is highest in the nucleolus, moderate in the cytoplasm, the nucleoplasm being poorly reactive. The nucleus has a central Feulgen-negative nucleolus and a peripheral Feulgen-positive chromatin area. Cysts, recently formed, resemble the trophic forms in their basophilia, alkaline phosphatase distribution and Feulgen reaction. Older cysts tend to show a drop in their substance localization after one week. Cysts, after three to four months, become completely negative to Feulgen reaction. Cysts become completely devoid of basophilia after four to five months. Drop in basophilia and alkaline phosphatase reactions in cysts starts from the cytoplasm and ends in the nucleolus. The cyst wall is Feulgen positive and is rich in alkaline phosphatase. It remains reactive to Feulgen till about two months after encystment and gives the reaction for alkaline phosphatase for about eight months after encystment. Older cysts, displaying weak basophilia and Feulgen reactions are capable of excystment. Animals which have encysted for 9 months and over cannot excyst; by then all indications of the presence of alkaline phosphatase are lost. The gradual depletion of RNA, DNA, alkaline phosphatase etc., would appear to be explainable on the basis of their utilization for the maintenance of life in absence of synthesis. The effect of prolonged encystment terminates the power of amoeba to excyst.

INTRODUCTION

The alternate existence of amoebae in trophic and cystic forms offers an important problem for investigation into their cytochemical nature. One of the earliest reports of obtaining living protozoa from old cysts is that of Gooday (1913). He obtained living *Colpoda* from dry museum specimens of soils. In 1915, he further showed that viable forms of *Amoeba* and small flagellates, viz., *Monas*, *Bodo* and *Circomonas* etc. could be obtained from forty-nine year old cysts from the same source. There is another report by Dawson and Hewitt (1931) on the viability of *Colpoda cucullus* which remained viable for more than five years in dry condition. Hausman (1934) obtained some living flagellates from materials dried for twenty years. Beers (1937) reported the viability of *Didinium nasutum* for ten years in sealed containers of hay infusion which did not withstand drying up.

Rafalko (1947) reported that cysts of *Negleria gruberi* could withstand drying, but he said nothing about their power of viability. In low temperatures, the sporozoites and asexual forms of human parasites remain viable for 375 days and 404 days respectively (Geoffroy and Rendtorff, 1955), though their viability limits have not been determined.

No serious attempt has yet been made to study the cytological and cytochemical changes associated with the process of transformation of trophic into cystic forms and *vice versa*. Such transformation of *Amoeba* from active life into resting stage is not merely a case of sol-gel conversion but should involve more complicated physiological changes. The present study is directed towards the understanding of the cytology and cytochemistry of *Amoeba* in trophic and encysted

conditions. To this end, tests for nucleic acids and enzymes like alkaline phosphatase were employed. The cytochemistry of the protoplasm of the cyst in course of prolonged encystment of the animal was also studied in relation to excystment-phenomenon.

MATERIAL AND METHODS

The material used was a species of soil amoeba, *Acanthamoeba sp.* presented by Dr. B. N. Singh of Lucknow to one of us (S. M.). The amoeba was subcultured by feeding it on *Aerobacter sp.* The *Aerobacter* also came from Lucknow and is now being maintained by us in the laboratory on nutrient agar slopes. •

Subcultures were made on slides by the technique developed by Singh (1950) with a slight modification. 0.05 percent NaCl solution in distilled water was used as culture solution. Cysts from old cultures were put on a clean slide in a drop of culture solution. A drop of thin suspension of *Aerobacter sp.* in saline was added. In 24 hours a perfect subculture of trophic forms was seen on the slide. Making of subcultures on agar was not found essential. The slides containing the subcultures were placed on wet filter paper in a large covered petri-dish. The cysts were manipulated by a micro-pipette with a length of rubber tubing attached to it. Cysts could be kept dry for long periods on the slides in a closed box and were subsequently tested for their survival by making fresh subcultures with *Aerobacter sp.* and culture solution.

For the detection of alkaline phosphatase, Gomori's modified technique was followed. Amoebae were fixed on the slide overnight in 80 per cent chilled alcohol. They were incubated at 37°C for half an hour.

For basophilia, the slides were fixed for three hours in Zenker's fluid and later washed overnight in tap water. They were then stained in 0.1 percent toluidine blue in 1 per cent alcohol for four minutes and quickly dehydrated and cleared before mounting.

To study the Feulgen reaction, the animals were fixed either in aceto-alcohol (Absolute alcohol-3 parts and acetic acid-1 part by volume) or in Carnoy's fluid for 3-5 hours. Optimum time for hydrolysis was found to be four minutes at 60°C.

OBSERVATIONS

CYTOCHEMISTRY OF NORMAL TROPHIC AMOEBA

Alkaline phosphatase :

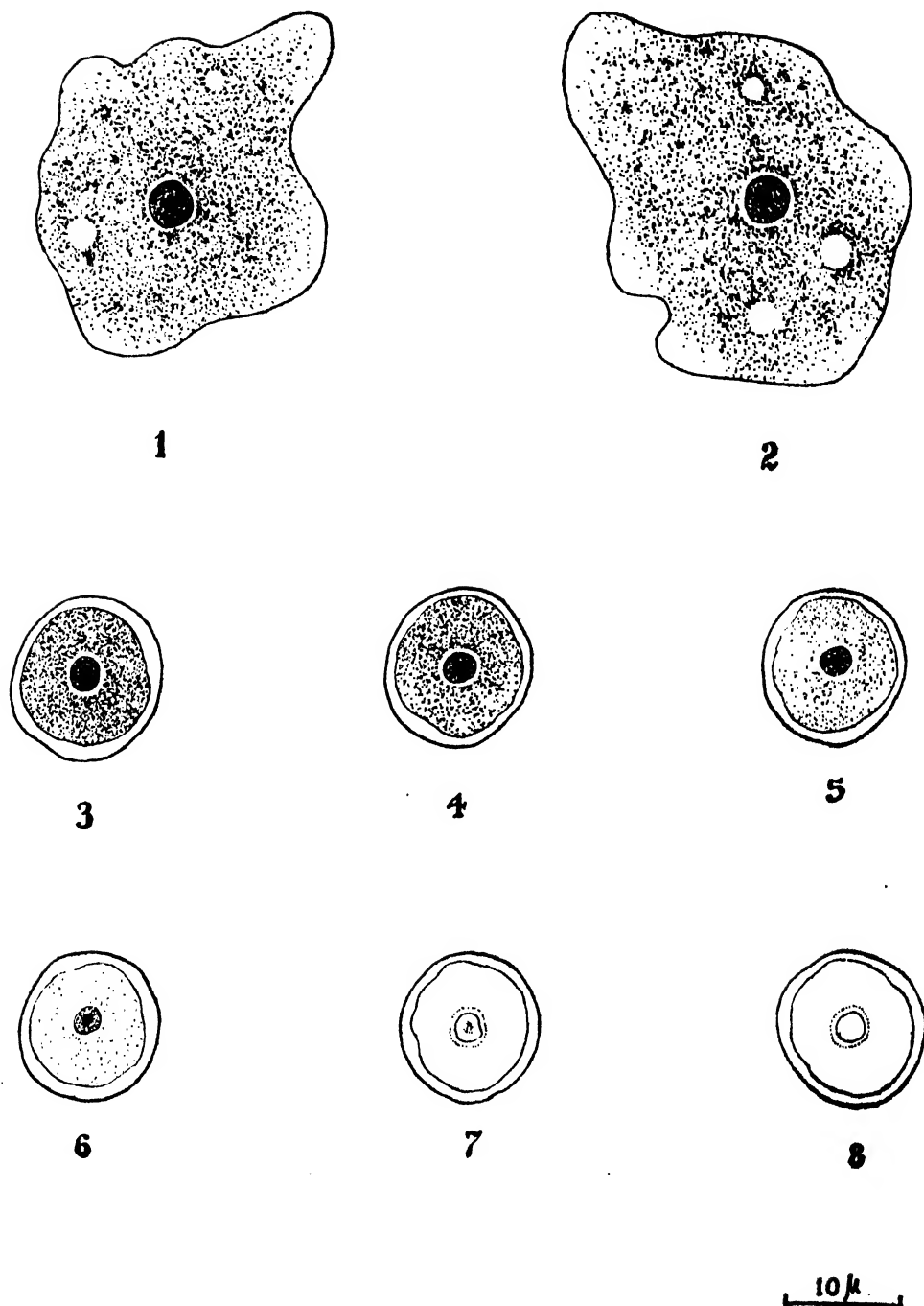
The alkaline phosphatase in the trophic forms of *Acanthamoeba sp.* from both new (i.e. two to three days old cysts) and old cysts (seven to eight months) shows a more or less uniform distribution. However, the intensity of reaction varies in the different regions of the animal (Text-fig. 1, fig. 1 and 2). The cytoplasm shows a moderate reaction, the nucleus a more intense one. The nucleolus seems to be the seat of highest phosphatase activity. In the cytoplasm, ectoplasm and endoplasm are clearly distinguishable. The ectoplasm shows no phosphatase reaction at all. The pseudopodia and contractile vacuoles are always negative. The plasma lemma is itself not reactive but can be clearly seen on the sides without pseudopodia. Vacuoles are seen as clear spaces in the cytoplasm.

Basophilia :

The cytoplasmic basophilia in the trophic forms obtained from new and old cysts is moderately homogeneous. The ectoplasm is negative and the pseudopodial bulge shows no basophilia. The nucleolus is deeply stainable but the nucleoplasm is not (Text-fig-2, fig. 9 and 10).

Desoxyribonucleic Acid :

The nucleus is Feulgen positive (Text-fig-3, fig. 18 and 19).



TEXT-FIG. I.

- Alkaline phosphatase localization in trophic and cystic forms of *Amoeba*
- | | |
|---|-----------------------------|
| Fig. 1—Normal trophic form. | Fig. 5.—1 month's old cyst. |
| Fig. 2—Trophic form obtained from old cyst. | Fig. 6—7 months' old cyst. |
| Fig. 3—2 days' old cyst. | Fig. 7—8 months' old cyst. |
| Fig. 4—7 days' old cyst. | Fig. 8—9 months' old cyst. |

CYTOCHEMISTRY OF ENCYSTED AMOEBA

Alkaline phosphatase :

2 days' old cyst.—The cytoplasm is moderately reactive. The nucleus shows a more intense reaction, the nucleolus displays the greatest activity. The cyst wall is also positive (Text-fig.-1, fig. 3).

7 days' old cyst.—The reaction is slightly weaker except in the cyst wall and nuclear area which present the same intensity of reaction (Text-fig.-1, fig. 4).

14 days' old cyst.—The reaction in the cytoplasm is very weak. The nucleus is positive. The reaction in the cyst wall is as before.

1 month old cyst.—A very faint reaction is shown by the cytoplasm. The nucleus and the cyst wall, however, continue to be rich in alkaline phosphatase (Text-fig.-1, fig. 5).

2 months' old cyst.—A very faint reaction is seen in the cytoplasm. The nucleus displays a weak reaction. The cyst wall, however, is positive as before.

4 months' old cyst.—The alkaline phosphatase reaction in the cytoplasm is almost negative. The nucleus shows loss of the phosphatase activity. The cyst wall continues to be positive.

7 months' old cyst.—The cytoplasm appears negative. The nucleus shows a very faint reaction. The nucleolus appears faintly positive. The cyst wall continues to show a considerable reaction (Text-fig.-1, fig. 6).

8 months' old cyst.—The entire cyst appears negative, except a very faint reaction in the nucleolus (Text-fig.-1, fig. 7).

9 months' old cyst.—No reaction for alkaline phosphatase is seen (Text-fig.-1, fig. 8).

Basophilia :

2 days' old cyst.—The nucleolus of the encysted amoeba shows the highest basophilia. The cytoplasm is positive but decidedly less than the nucleolus. No basophilic reaction is observed in the cyst wall (Text-fig.-2, fig. 11). The basophilia in pre-cystic forms, is significantly high (Text-fig.-2, fig. 12).

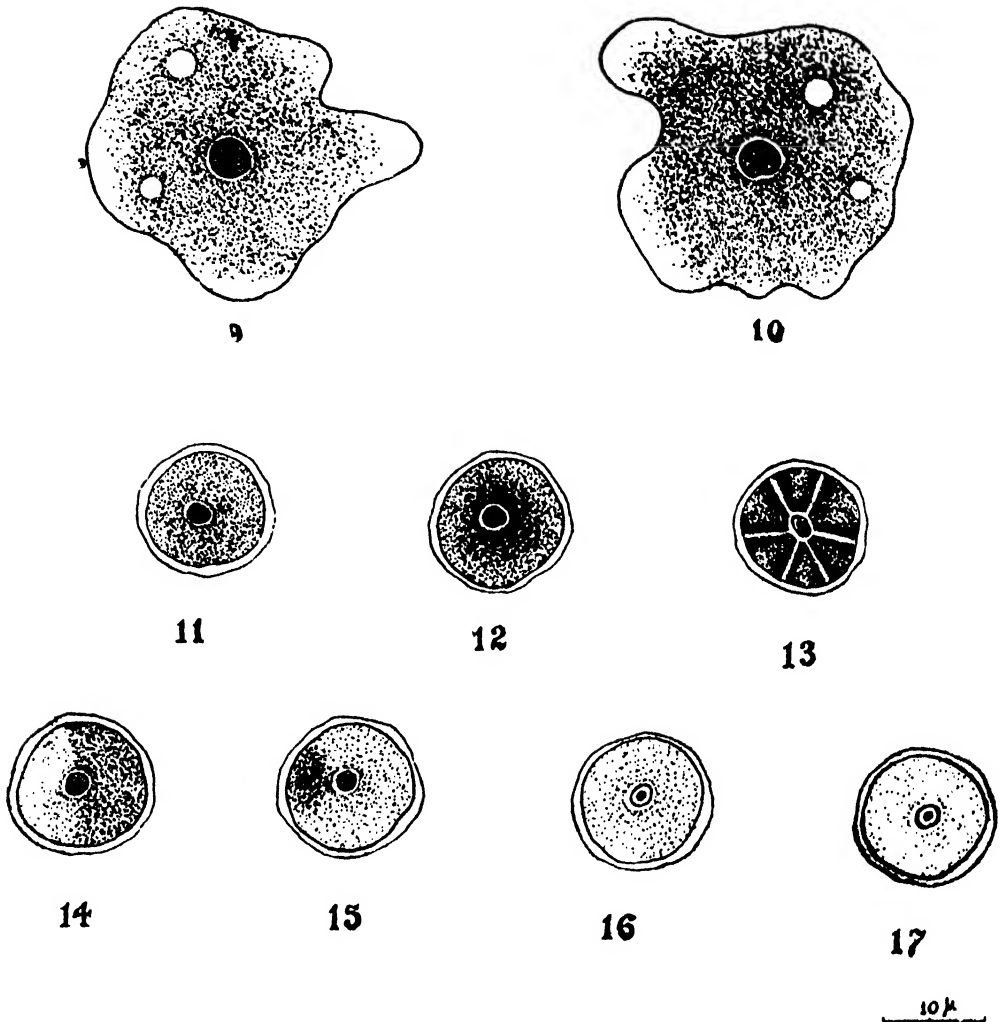
14 days' old cyst.—The intensity of reaction in the cyst shows a tendency to fall. The nucleolus continues to be intensely basophilic. Some of the cysts encountered, show the furrowing of the cytoplasm into sectors. (Text-fig.-2, fig. 13). The conspicuous feature of such cysts is that the basophilic reaction is more intense at the line of the furrows than in the middle of the cytoplasm. There are some other cysts which show that the negative basophilic space has filled up a great portion of them. (Text-fig.-2, fig. 14). Majority of the cysts are lowly reactive round ones.

2 months' old cyst.—A further loss of basophilia in the nucleus and cytoplasm is seen. In some cysts, a region is sometimes observed which shows slightly higher basophilia than in the rest of the cytoplasm (Text-fig.-2, fig. 15)). The wall of the cyst appears basophilic.

4 months' old cyst.—The majority of cysts has lost basophilia (Text-fig.-2, fig. 16). The cyst wall shows basophilia. In some cysts, the basophilia is more intense in the peripheral region than in the central region. The nucleolus is moderately positive and the cyst wall shows no reaction.

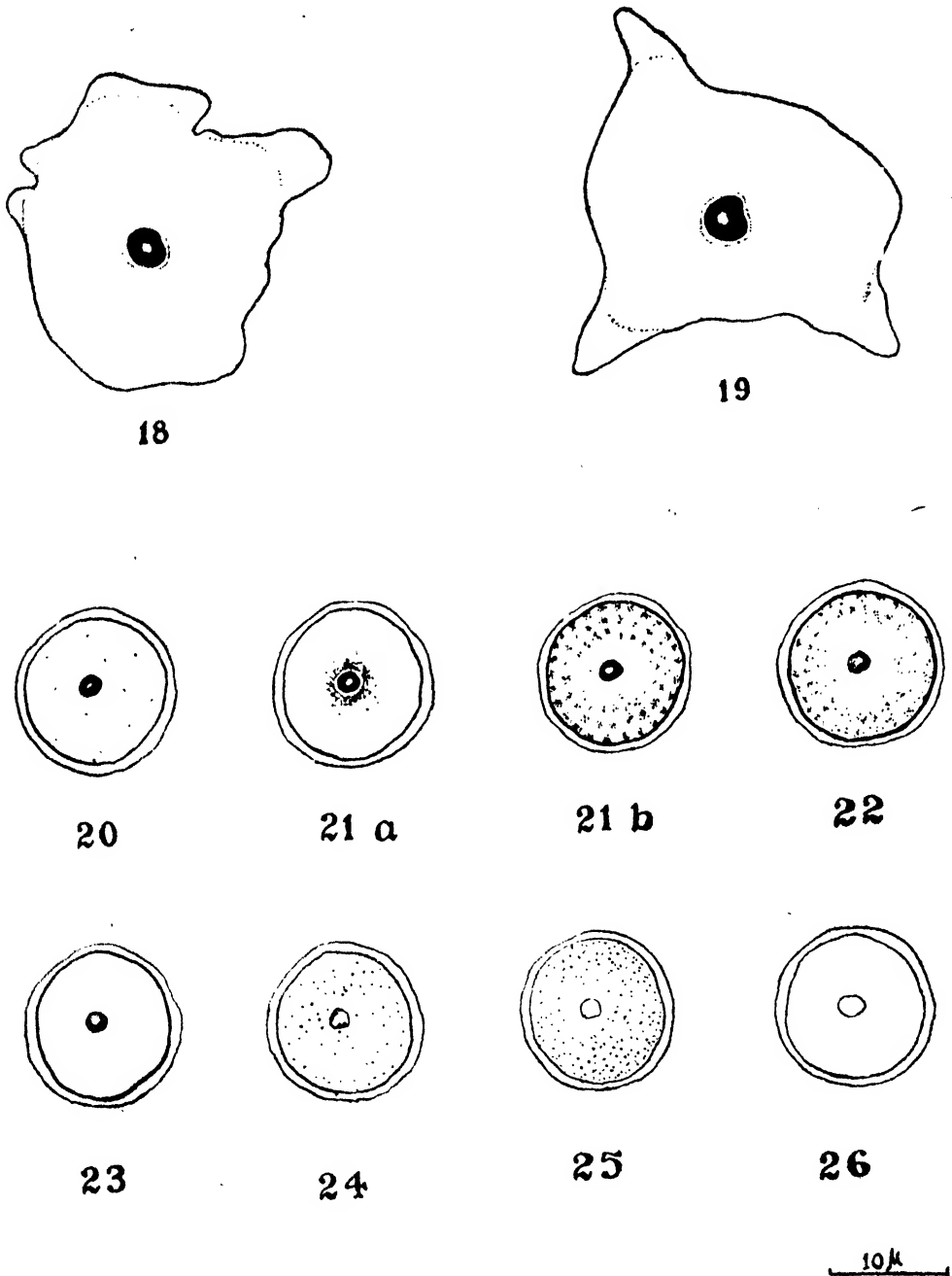
5 months' old cyst.—Cysts show no basophilia. The cyst wall, however, continues to exhibit basophilia (Text-fig.-2, fig. 17).

Cysts over 5 months' old.—Cysts are devoid of basophilia. Toluidine blue does not render the details of the cytoplasm or the nucleus visible. The reaction in the cell wall also becomes gradually feeble with age of the cyst.



TEXT-FIG. 2.

- Basophilic concentration in trophic and cystic forms of *Amoeba*
- | | |
|--|-----------------------------|
| Fig. 9—Normal trophic form. | Fig. 13—14 days' old cyst. |
| Fig. 10—Trophic form obtained from old cyst. | Fig. 14—14 days' old cyst. |
| Fig. 11—2 days' old cyst. | Fig. 15—2 months' old cyst. |
| Fig. 12—Precystic form. | Fig. 16—4 months' old cyst. |
| | Fig. 17—5 months' old cyst. |



TEXT-FIG. 3

Feulgen reaction in trophic and cystic forms of Amoeba
 Fig. 18—Normal trophic form.
 Fig. 19—Trophic form obtained from old cyst.
 Fig. 20—2 days' old cyst.
 Fig. 21a, 21b and 22—7 days' old cyst.
 Fig. 23—21 days' old cyst.
 Fig. 24—35 days' old cyst.
 Fig. 25—2½ months' old cyst.
 Fig. 26—3 months' old cyst.

Desoxyribonucleic Acid:

2 days' old cyst.—In the cytoplasmic area, there are certain bodies which are stainable by Feulgen stain. Nucleus shows a positive reaction (Text-fig.-3, fig. 20). The cyst-wall appears to be Feulgen-positive.

7 days' old cyst.—The inside area of the nucleus shows a less intense DNA reaction (Text-fig.-3, fig. 21a). Some amount of Feulgen positive material is seen outside the nuclear membrane. This condition is seen in most cysts. In some cysts, the nucleus indicates more red reaction. Cyst-wall of these cysts shows red colouration, and in the cytoplasm, some granules, oriented mostly at the periphery, take up yellowish pink colouration more brightly (Text-fig.-3, fig. 21b). Cysts, with crumpled walls, show reaction in the inner side of the cyst-wall; the outer wall remains unstained. Cysts, with smooth walls, have Feulgen-positive cytoplasmic bodies. Sometimes these granules show a deep reaction, more intense than the nucleus (Text-fig.-3, fig. 22).

21 days' old cyst.—A faint reaction in the nucleus is seen (Text-fig.-3, fig. 23). It is seen on the inner side of the cyst-wall also. Yellow-pink granules outside the nuclear membrane are no longer visible.

35 days' old cyst.—The nucleus appears nonstainable (Text-fig.-3, fig. 24). In some cysts, however, faintly Feulgen-positive granules are found in the cytoplasm, but the nucleus remains as a nonstainable body.

2 months' old cyst.—The nucleus is faintly visible. Inner side of the cyst wall may show, at times, a positive reaction. The cytoplasm shows some positive reaction. The outer cyst-wall is non-reacting.

2½ months' old cyst.—No positive reaction is found in the protoplasm. The nucleus does not react with the Feulgen technique. In some cysts, however, faintly positive granules continue to be seen in the cytoplasm (Text-fig.-3, fig. 25).

3 months' old cyst.—No reaction is seen either in the nucleus or in any other region of the cyst (Text-fig.-3, fig. 26).

Over 3 months' old cyst.—Ameobae, remaining encysted for over 3 months, become completely Feulgen-negative.

DISCUSSION

This study portrays the occurrence and distribution of three important substances in the protoplasm, of the active trophic and inactive cystic forms of Amoeba. Trophic forms grow by synthesizing new materials within their cell-body. The cystic forms, however, live on the material already synthesized inside them.

With prolonged encystment, the cytoplasmic RNA in the cysts gradually becomes depleted. The nuclear basophilia takes a much longer time for its disappearance. The paucity of cytoplasmic RNA in an inactive cell is understandable in the light of researches by Brachet (1942) and Caspersson (1947). The RNA is always present in rich quantity in sites of active synthesis. That RNA is involved in protein synthesis has recently been shown experimentally by Prescott and Mazia (1954). Evidently Amoebae in cystic condition are not engaged in fresh synthesis, and whatever RNA is present in the cytoplasm is being utilized in the cyst. James (1954) has recently presented evidence that the nucleus is responsible for maintaining the level of RNA in the cytoplasm. It is also on record that in intact normal tropic forms of Amoebae, starvation results in a drop of RNA (Prescott and Mazia, 1954; James, 1954). Singh (1952) has studied the nuclear apparatus in Amoebae by the Feulgen technique. According to him, there is a central Feulgen-negative nucleolus and a peripheral Feulgen positive chromatin area. In the newly formed cyst, the same condition of nucleus is seen. However, prolonged encystment has a decided influence on the subsequent stainability of its nucleus.

After seven days of encystment, the nuclear stainability becomes further weakened. The nucleus appears almost entirely negative after 3 months of encystment.

The reaction for alkaline phosphatase is significantly different from other reactions. The alkaline phosphatase becomes progressively lowered with the age of the cyst, but is not rendered negative so quickly.

The viability of the cysts and their staining reactions appear to be related. It has been shown that basophilia and the Feulgen and alkaline phosphatase reactions suffer with encystment. However, even cysts with markedly less stainability excyst and produce trophic forms when subcultured before too long an encystment has occurred. These trophic forms present normal cytochemical localization. The viability of the cysts is completely lost after nine months of encystment. The interesting point is that alkaline phosphatase continues to be present in the cyst till the last, whereas basophilia and the Feulgen reaction are obscured after 4 months and 3 months of encystment respectively. It would appear that the viability of the cyst is associated rather intimately with the presence of alkaline phosphatase and as long as the latter is present the cyst continues to be viable.

Another interesting aspect of this study relates to the staining reactions in the cyst wall. The surface of a trophic Amoeba is negative to Feulgen and shows no basophilia and alkaline phosphatase. However, soon after cyst formation, the cyst wall, unlike other parts of the cyst, becomes progressively positive for all these three. Some Feulgen-positive bodies are seen in the cytoplasm soon after encystment. Very likely, they are of nuclear origin and tend to migrate towards the cyst wall. The true nature of such bodies is uncertain. Ray and Sengupta (1954) observed some dust like Feulgen positive particles in the endoplasm of *Entamoeba histolytica* and reported that the Feulgen positive chromatoid bodies contain varying amounts of DNA and RNA. Barker and Deutsch (1958) made some histochemical studies and said that the chromatoid bodies in *Entamoeba invadens* consist mainly of ribonucleic acid and some unspecified proteins. Their preliminary electron microscope study has shown that the globular particles which form these bodies are flattened after treatment with ribonuclease. But the fact remains valid that after some period of encystment these particles become no longer visible; very naturally they are utilized during encystment. The cyst nucleus becomes Feulgen-negative after 2½ months of encystment, but the cyst wall continues to be Feulgen positive, exhibits basophilia and is rich in alkaline phosphatase. This positive reaction on the inner side of the cyst wall is difficult to explain. It suggests that this part of the cell may not be altogether a dead secretion, and if so, it may be associated with vital function for the existence of the cyst.

Recently the interaction between the nucleus and cytoplasm in trophic Amoebae has become the subject of intense work (Danielli, *et al.*, 1955; Mazia and Prescott, 1955; Brachet, 1957). The general trend of the findings is that both nucleus and cytoplasm influence each other in a reciprocal manner. Specially, the nuclear origin of cytoplasmic RNA has been shown in experiments with isotopes (Goldstein and Plaut, 1955). This has led Brachet to express the view that the nucleus exerts an important control on the maintenance of cytoplasmic RNA. In the present investigation, it is seen that the nucleus in encystment is not capable of providing RNA to the cytoplasm. Brachet (1957) has expressed further that "inhibition of RNA synthesis leads to an inhibition of protein synthesis, but reverse is not true. It is to be concluded that protein synthesis is dependent upon RNA synthesis" (p. 251). In encysted forms of Amoebae where protein synthesis is not continuing, cytoplasmic RNA becomes progressively reduced.

The life of the cyst, however, cannot continue for an indefinite period. It varies within rather narrow limits and depends on the materials already accumulated in the cell. For the species studied here, the period appears to be about nine months. This period may also vary with the species, because there are previous records of viability of cysts of other species for longer periods. A few tests

for glycogen in the cysts have also been made (Mookerjee and Hajra, *unpublished*). The glycogen test of the cysts runs almost parallel with other tests, i.e., progressively the glycogen stainability drops with period of encystment, until a time comes when the cyst renders glycogen stain impossible. The time of depletion almost coincides with the alkaline phosphatase localization. The most important point is that the energy for the maintenance of an inactive cell is sustained by the material already present in the encysted cell. It is well known from the researches of Mazia and Prescott (1955) that the nucleus is a centre of protein synthesis but in dehydrated condition of the protoplasm it is ineffective. The different metabolic processes are facilitated by diffusion and in prolonged encystment they become ineffectual. The only significant function that the cell is capable of performing in inactive phase is the carrying out its basic metabolism. In the life of an encysted amoeba a stage comes when the material inside the encysted cell is utilized and the life of the cyst is terminated. Mazia's (1952) assumption seems valid not only in trophic condition of *Amoeba* but also in cystic condition that 'replacing' is necessary between the nucleus and the cytoplasm for the proper maintenance of life. The study of the phase between the trophic and cystic forms has abundantly shown that.

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NITROGEN FIXATION BY *AZOTOBACTER* IN ASSOCIATION WITH SOME ASSOCIATED SOIL MICROORGANISMS*

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ABSTRACT

In order to find out the effect on nitrogen fixation by *Azotobacter* when grown in association with various soil microorganisms, four strains of *Azotobacter*, belonging to two species, *A. chroococcum* and *A. agile*, isolated from irrigated soils of the Agricultural College Farm, Poona, were cultivated singly with soil bacteria, including some *Rhizobium* spp., and actinomycetes in mixed cultures in test-tubes containing 10 ml. of Ashby's mannitol-phosphate solution to which molybdenum and iron were added. Total nitrogen was determined after 15 days incubation at 28°C. None of the bacterial cultures, except four strains of *Rhizobium* spp., stimulated nitrogen fixation in case of all the four *Azotobacter* strains. Some actinomycetes stimulated nitrogen fixation while others depressed it. Four strains of *Rhizobium* spp. greatly stimulated nitrogen fixation. There is thus a possibility that legumes might have a stimulatory effect on nitrogen fixation by *Azotobacter* cells in the close proximity of the legume root.

INTRODUCTION

The genus *Azotobacter* has been extensively studied since its discovery by Beijerinck in 1901. It was thought that a study of the effect on nitrogen fixed by *Azotobacter* when grown in association with other soil microorganisms would be interesting since this aspect has not been yet thoroughly investigated.

HISTORICAL

Markinow (1934) reported that when grown in association with cellulose decomposing bacteria, nitrogen fixation by *Azotobacter* was stimulated. Richards (1939) found that a strain of *Azotobacter chroococcum* fixed more nitrogen when grown in association with a capsulated organism of the *Aerobacter aerogenes* type. Jensen (1940) noticed a significant increase in nitrogen fixation when *Azotobacter* was cultivated together with *Cellulomonas blazotea*; he, however, found no increase in the amount of nitrogen fixed when the associating microorganisms were members of the genera *Cytophaga* and *Cellvibrio*. Perhaps the most extensive investigation on this problem was conducted by Lind and Wilson (1942). They found that a culture of *Azotobacter vinelandii*, contaminated by an aerobic spore former, tentatively identified as *Bacillus circulans*, fixed considerably more nitrogen than *A. vinelandii* alone. They further experimented on associations of various other microorganisms including some *Rhizobium* spp. and reached the conclusion that with the exception of *Clostridium pasteurianum*, which fixes nitrogen by itself anaerobically, no other microorganisms studied stimulated nitrogen fixation. Rybalkina (1949) observed that microorganisms isolated from peat showed varying response when grown in association with *Azotobacter*; some were stimulatory, some antagonistic while others

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were without any effect. Waksman (1952) reported that *Rhizobium leguminosarum* stimulated nitrogen fixation by *Azotobacter*. Nickell and Burkholder (1947) found 25 cultures of actinomycetes that either suppressed the growth or killed out the *Azotobacter* cells when the two were grown together. Lall and Achari (1953), on the other hand, reported that *Azotobacter* in association with certain actinomycetes fixed more nitrogen than when grown alone.

MATERIAL AND METHODS

Using the enrichment culture method, four strains of *Azotobacter* were isolated from irrigated soils of the Agricultural College Farm, Poona. The medium used was Ashby's mannitol-phosphate solution; 100 ml. lots of this solution were placed in 500 ml. Erlenmeyer flasks and inoculated with 1 gram soil samples. Incubation was carried out at room temperature (28°-30°C.). Within a week a brownish pellicle developed on the surface of the medium in most cases. From this pellicle streaks were made on Ashby's agar plates and transfers to Ashby's agar slants were made from well isolated colonies that developed on the streaked plates. The four *Azotobacter* strains thus isolated were designated A, B, C and D. They differed in pigment production; strain A produced a deep brown pigment, strain D produced a very light brown pigment, strains B and C produced pigments intermediate between these two extremes. The morphological and physiological characters of these strains were studied and strains A and C were identified as *Azotobacter agile*, Beijerinck and strains B and D as *Azotobacter chroococcum*, Beijerinck. Cultures of soil bacteria and actinomycetes were obtained from the irrigated soils of the Agricultural College Farm, Poona, using the dilution and plating technique. Dilutions from 1 in 1,000 to 1 in 1,00,000 were plated using Ashby's agar and transfers from well isolated colonies were made on Ashby's agar slants. In all, 11 bacterial cultures and 14 actinomycetes cultures were isolated in this manner. Morphological and physiological characters of these cultures were studied and some of them could be identified as noted below :

- Culture no. 4 : *Cytophaga* spp.
- Culture no. 9 : *Aerobacter aerogenes*
- Culture no. A 1 : *Streptomyces coelicolour*
- Culture no. A 2 : *S. coelicolour*,
- Culture no. A 3 : *Streptomyces* spp.
- Culture no. A 4 : *Streptomyces* spp.
- Culture no. A 6 : *S. rutgersensis*,
- Culture no. A 7 : *Streptomyces* spp.
- Culture no. A 8 : *S. griseus*,
- Culture no. A 10 : *S. albus*,
- Culture no. A 11 : *Streptomyces* spp.
- Culture no. A 12 : *Streptomyces* spp.
- Culture no. A 13 : *Micromonospora globosa*
- Culture no. A 17 : *Streptomyces albus*
- Culture no. A 18 : *Streptomyces* spp.
- Culture no. A 20 : *Streptomyces* spp.

Three strains of *Rhizobium leguminosarum* isolated from pea and lentil and one strain of *Rhizobium* spp. of the cowpea group isolated from cowpea from the stock cultures maintained by the Plant Pathological Laboratory, College of Agriculture, Poona, were also used in these experiments.

An *Azotobacter* strain and a soil microorganism were cultivated together in triplicate test-tubes, each containing 10 ml. of Ashby's mannitol-phosphate solution. Four p.p.m. of molybdenum and 0.05 gm. per litre of ferric chloride were added to the Ashby's solution since these elements are shown to be essential for proper growth of *Azotobacter*. (Burema and Wieringa, 1942; Horner, Burk, Allison

and Sherman, 1942). The pH of this solution was adjusted to 7.2. The tubes on inoculation were incubated at 28°C. for 15 days, at the end of which period the whole solution along with the culture growth was used for nitrogen estimation by the Kjeldahl method. For quicker digestion, the rapid wet digestion micro-method outlined by Pepkowitz and Shive (1942) was followed.

RESULTS

The amount of nitrogen (in milligrammes per ten millilitres of the medium) fixed by strains A, B, C and D of *Azotobacter* in association with bacterial cultures 1, 2, 3, 4, 5, 8, 9, 11, 12, 14 and 15, actinomycetes cultures A 1, A 2, A 3, A 4, A 6, A 7, A 8, A 10, A 11, A 12, A 13, A 17, A 18 and A 20, *Rhizobium leguminosarum* cultures M 1, M 2 and P 7 and *Rhizobium* spp. (cowpea group) culture A1 3, is recorded in Table I. Each value represents average of three replications. Control represents the amount of nitrogen fixed by that strain of *Azotobacter* when it is grown alone.

The data were statistically treated and the results in all four cases were found to be significant.

TABLE I
Amount of Nitrogen fixed by *Azotobacter* strains A, B, C and D in association with soil Bacteria and Actinomycetes
(Averages of three replications)
Mgm. of nitrogen per 10 ml.

Associating microorganisms	<i>Azotobacter</i> strains			
	A	B	C	D
Control	0.205	0.200	0.238	0.274
Culture no. 1	0.119	0.132	0.201	0.257
Culture no. 2	0.097	0.177	0.151	0.205
Culture no. 3	0.079	0.092	0.173	0.155
<i>Cytophaga</i> spp.	0.305	0.245	0.236	0.325
Culture no. 5	0.265	0.214	0.182	0.191
Culture no. 8	0.201	0.150	0.218	0.218
<i>Aerobacter aerogenes</i>	0.265	0.258	0.204	0.110
Culture no. 11	0.213	0.160	0.175	0.198
Culture no. 12	0.209	0.223	0.165	0.165
Culture no. 14	0.091	0.129	0.090	0.099
Culture no. 15	0.108	0.156	0.104	0.143
<i>Streptomyces coelicolor</i>	0.161	0.285	0.246	0.277
<i>Streptomyces coelicolor</i>	0.345	0.443	0.324	0.355
<i>Streptomyces</i> spp.	0.355	0.470	0.463	0.408
<i>Streptomyces</i> spp.	0.361	0.370	0.370	0.509
<i>Streptomyces rutgersensis</i>	0.016	0.013	0.060	0.072
<i>Streptomyces</i> spp.	0.243	0.259	0.293	0.213
<i>Streptomyces griseus</i>	0.269	0.269	0.301	0.199
<i>Streptomyces albus</i>	0.295	0.236	0.264	0.338
<i>Streptomyces</i> spp.	0.393	0.436	0.412	0.441
<i>Streptomyces</i> spp.	0.482	0.547	0.588	0.509
<i>Micromonospora globosa</i>	0.423	0.379	0.361	0.384
<i>Streptomyces albus</i>	0.249	0.269	0.311	0.246
<i>Streptomyces</i> spp.	0.160	0.194	0.246	0.213
<i>Streptomyces</i> spp.	0.319	0.283	0.292	0.259
<i>Rhizobium leguminosarum</i>				
strain M 1	0.528	0.496	0.556	0.551
strain M 2	0.681	0.662	0.695	0.648
strain P 7	0.454	0.417	0.491	0.459
<i>Rhizobium</i> spp. strain A1 3	0.574	0.565	0.625	0.607

CONCLUSION

The results presented in the table show that, with the notable exception of *Rhizobium* spp., the bacterial cultures under study did not stimulate nitrogen fixation greatly. Six actinomycetes cultures, viz., A 2, A 3, A 4, A 11, A 12 and A 13 did stimulate nitrogen fixation in case of all the four *Azotobacter* strains. However, considerable increase in nitrogen fixation was noticed in case of all the four strains of *Azotobacter* when they were grown in association with strains of *Rhizobium* spp. The gain in soil nitrogen which unquestionably takes place when leguminous plants are grown is usually attributed to nodules of the legumes left to decompose in the soil and to the nitrogenous products excreted by the nodules. It appears probable that the stimulation of nitrogen fixation by *Azotobacter* when grown in association with *R. leguminosarum* and other *Rhizobium* spp. which occurs to such a great extent under laboratory conditions (a fact also recorded by Waksman, 1952) might also occur under natural conditions in the soil with the result that *Azotobacter* cells in the rhizosphere of legumes fix much greater quantities of nitrogen than they would in the absence of a legume crop. The gain in soil nitrogen in such a case would then be attributable, not to the legume crop alone, but also in part to *Azotobacter* activity. To substantiate such a hypothesis, pot and field tests are of course quite essential, but the present work might be taken to indicate that such an hypothesis is not unwarranted.

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STUDIES ON THE OXYGEN CONSUMPTION IN TROPICAL POIKILO- THERMS. IV. OXYGEN CONSUMPTION IN THE FRESH WATER FISH, *PUNTIUS SOPHORE* (HAMILTON) IN RELATION TO SIZE AND TEMPERATURE

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ABSTRACT

In *Puntius sophore* increase in oxygen consumption with increasing body weight, as indicated by the regression coefficient of metabolism against size, is maximal at the habitat temperature and less so at other temperatures, higher as well as lower. Hence the b value of the size-metabolism curves is maximal at 25°C. Consequently the rate of decrease in the unit metabolism with increasing body size exhibits a parallel dependence on temperature. The smaller fish appear to be more sensitive to temperature changes (as measured by Q_{10}) than the larger ones, but the latter show marked heat depression at 35°C and indications of cold depression at 15°C. With increasing body weight the Q_{10} increases at temperatures below the habitat temperature and decreases at temperatures above the habitat temperature. The Q_{10} values are less size dependent at lower temperatures than at the higher. The Q_{10} values do not show any systematic temperature trend except in the 16 gm. fish, where the Q_{10} systematically decreases with the increasing temperature. This appears to be associated with its heat depression at 35°C. Highest Q_{10} value is noted in the smallest fish at the highest temperature range and in the largest fish at the lowest temperature range.

INTRODUCTION

The influence of body size and environmental temperature on metabolism, as measured by the oxygen consumption, has already been described by the author in a tropical fresh water fish, *Etroplus maculatus* (Parvatheswararao, 1959). To determine whether any generalisation can be made regarding the influence of body size and environmental temperature on the metabolism and activity of tropical poikilotherms, the investigations have been extended to another tropical fresh water fish, *Puntius sophore* and these are described in the present communication.

MATERIALS AND METHODS

The fish were collected from the local fresh water ponds and stocked in the laboratory in large aquaria along with *Etroplus maculatus*. The fish were fed regularly once in two days, and water in the aquaria renewed once a week. Feeding metabolism was measured in specimens of different body weights at different temperatures, like 15°C, 25°C, 30°C, and 35°C. 25°C was the approximate acclimated temperature, as the temperature of the aquaria varied between 24 and 27°C during the period of these studies. The procedure of these experiments was the same as reported in an earlier work (Parvatheswararao, 1959), and the estimation of oxygen content in the water samples was by the Winkler's iodometric method as described in Welsh and Smith (1953). The experimental duration was 15 minutes in each case and the fish were found to be somewhat slimy.

RESULTS

The results obtained are presented in the accompanying Tables and also plotted in the several graphs.

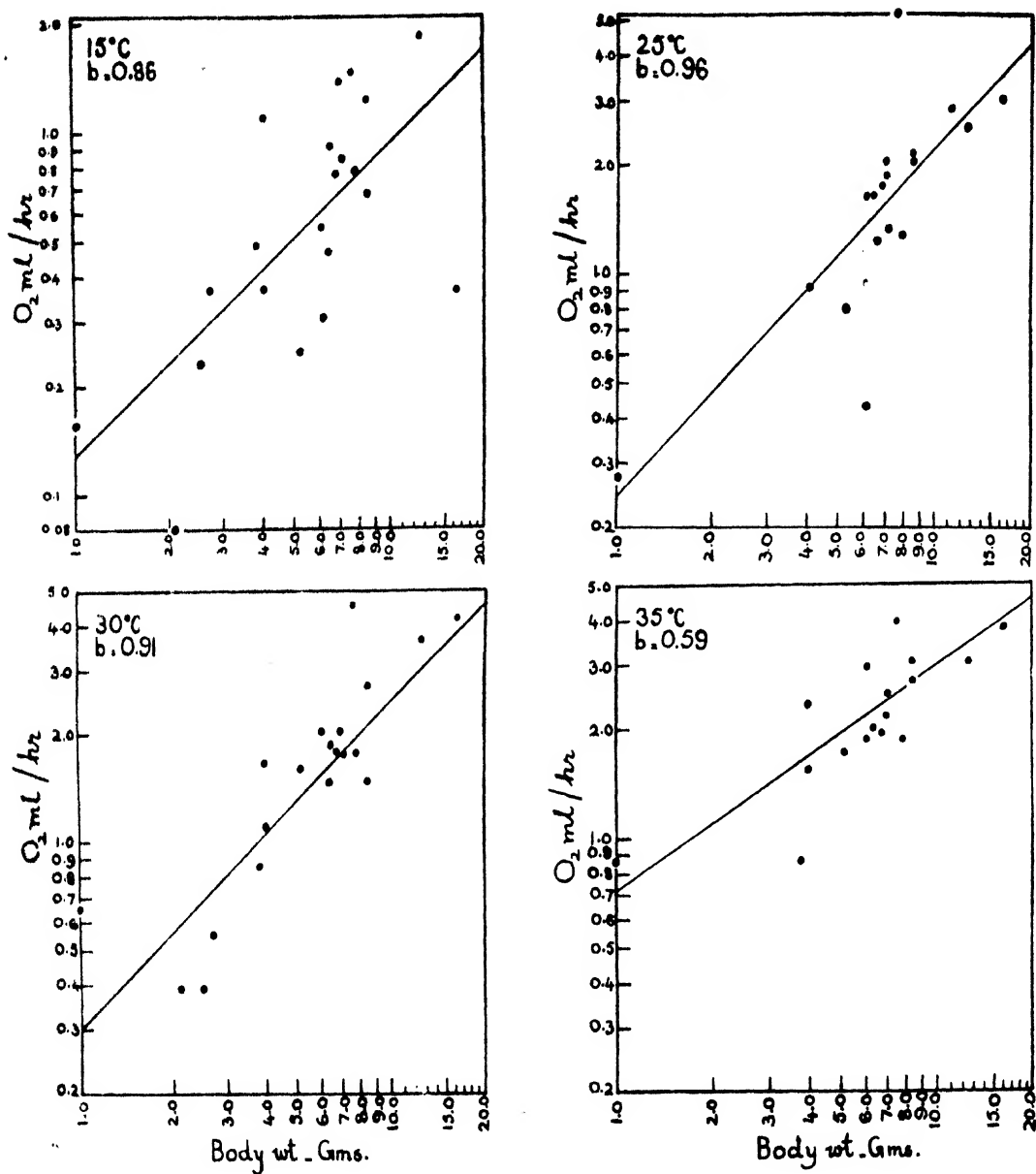
TABLE 1

Total oxygen consumption in Puntius sophore of different body weights at different temperatures

Serial number	Weight of fish Gms.	O ₂ ml./hr.			
		15°C	25°C	30°C	35°C
1	1.0	0.154	0.271	0.658	0.851
2	2.1	0.080	...	0.390	...
3	2.5	0.230	...	0.390	...
4	2.7	0.370	...	0.550	...
5	3.8	0.490	...	0.860	0.860
6	4.0	0.370	...	1.100	1.530
7	4.0	1.100	0.920	1.650	2.330
8	5.2	0.250	0.800	1.590	1.710
9	6.1	0.550	0.430	0.370	2.940
10	6.1	0.310	1.630	2.010	1.860
11	6.4	0.470	1.630	1.470	2.010
12	6.5	0.920	1.220	1.840	...
13	6.8	0.770	1.740	1.780	1.940
14	7.0	1.390	2.010	2.010	2.170
15	7.0	...	1.860
16	7.1	0.850	1.320	1.740	2.480
17	7.6	1.470	5.190	4.530	3.920
18	7.8	0.780	1.280	1.780	1.860
19	8.5	0.680	2.020	1.470	3.060
20	8.5	1.230	2.140	2.690	2.690
21	11.2	...	2.820
22	12.6	1.840	2.510	3.610	3.060
23	16.2	0.370	3.000	4.160	3.800

Oxygen consumption as a function of body size :

The values for the total oxygen consumption of the fish (O₂ ml./hr./fish) of various body weights at different temperatures are shown in Table 1 and plotted as size-metabolism curves in Fig. 1. At all the temperatures studied the oxygen consumption increases with increasing body weight of the fish, but there are clear indications of the existence of different responsive patterns by fish of different body sizes to these temperature changes as evidenced by the regression coefficients of the size-metabolism curves (Fig. 1 and Table 1). The regression coefficient of the oxygen consumption in relation to body size is maximal at 25°C and decreases at higher or lower temperatures. It is of interest that 25°C is the mean acclimation temperature of the fish. Further, throughout the weight range of the fish studied, the oxygen consumption increases with the same power of the body weight as is suggested by the straight lines of the size-metabolism curves. Unit oxygen consumption (O₂ ml./gm./hr.) values for fish of representative weights, calculated from the curves of Fig. 1 are presented in Table 3. The unit oxygen consumption decreases with increasing body weight of the fish at all temperatures studied. Such a decrease in unit oxygen consumption with increasing body weight at various



TEXT-FIG. 1.

Total oxygen consumption of *Puntius sophore* as a function of body size at different temperatures, plotted on double logarithmic grid. The points in the figure represent individual measurements. The experimental temperature and the regression value are indicated in relation to the corresponding curve.

temperatures reflects the temperature trends of the size-metabolism curves, already pointed out.

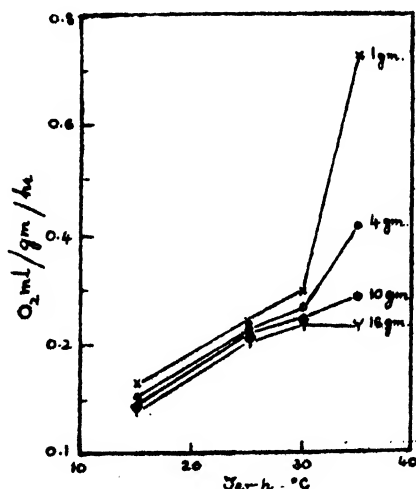
TABLE 2

The regression coefficient of oxygen consumption in relation to body size in Puntius sophore at different temperatures

Temperature	15°C	25°C	30°C	35°C
*Regression coefficient	0.86	0.96	0.91	0.59

Oxygen consumption as a function of temperature :

It may be noted that the size-metabolism curves of Fig. 1 have different b values at different experimental temperatures (Table 2). Thus at 15°C the b value of the curve is 0.86, at 25°C it is 0.96, at 30°C it is 0.91, and at 35°C 0.59. Such a variation of the b indicates that the increase in the oxygen consumption with the increasing body size is not the same at all temperatures. The unit oxygen consumption values of the fish of representative weights presented in Table 3, are plotted in Fig. 2 as Rate-Temperature curves. An examination of these curves will reveal that the body size is a very important parameter in influencing the pattern of metabolic response of the fish to the varying temperatures. The smaller fish are not only sensitive to these temperature changes but appear to be more tolerant of the same. Unlike this the larger fish, like the 16gm. one, show marked heat depression at 35°C and indications of cold depression as well at 15°C. The same can be made out from the Q_{10} values discussed below. It may further be noted from Table 3 that the extent of metabolic response to temperature changes is directly proportionate to the temperature gradient encountered by the fish. Thus the unit oxygen consumption values of various fish vary much more between 25 and 35°C or 25 and 15°C than between 25 and 30°C. The only exception to



TEXT-FIG. 2.

Rate of oxygen consumption (O_2 ml./gm./hr.) as a function of temperature in *Puntius sophore* of 1, 4, 10 and 16 gm. weights. The points are taken from the size-metabolism curves of Figure-1.

this trend is the lesser variability in the unit oxygen consumption of a 16 gm. fish between 25 and 35°C than between 25 and 30°C and this is associated with the heat depression in this fish at 35°C.

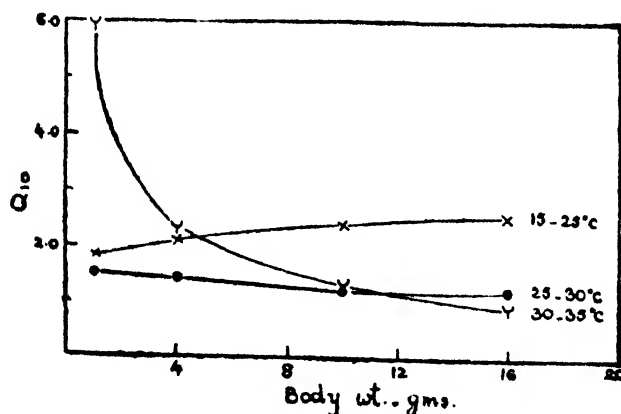
TABLE 3

Oxygen consumption per gram of body weight per hour in *Puntius sophore* of different sizes at different temperatures. The values are calculated from the size-metabolism curves presented in Figure-1

Weight of fish Gms.	O ₂ ml./gm./hr.			
	15°C	25°C	30°C	35°C
1	0.120	0.240	0.297	0.724
4	0.107	0.224	0.268	0.413
10	0.092	0.219	0.246	0.282
16	0.086	0.212	0.238	0.232

Q_{10} as a function of body size and temperature :

The values for the Q_{10} of oxygen consumption in *Puntius sophore* were calculated from the data presented in Table 3. The same are presented in Table 4 and plotted as curves in Fig. 3. To a greater or lesser extent the Q_{10} values in *Puntius sophore* are size dependent at all temperatures. At low temperature range (15-25°C) they increase with the increasing body weight and at higher temperature ranges (25-30°C and 30-35°C) they decrease with the increasing body weight. Such a decrease is less marked and systematic at the lower of the two higher temperature ranges. Thus at 25-30°C range the Q_{10} decreases from 1.54 to 1.25 between 1 and 16 gm. fish, while the corresponding decrease between the same fish at 30-35°C range is from 5.95 to 0.97. Further at all temperature ranges the variability of the Q_{10} values with the body weight is the steepest and most marked in smaller specimens than in the larger ones, testifying to the greater degree of size dependence of the Q_{10} values in the smaller fish than in the larger ones (Table 4).



TEXT-FIG. 3.

Q_{10} of oxygen consumption in *Puntius sophore* as a function of body size, at different temperature ranges. The Q_{10} values are calculated from the unit oxygen consumption data shown in Table-3.

TABLE 4

Q_{10} of oxygen consumption in *Puntius sophore* in relation to body size and temperature. The Q_{10} values are calculated from the unit oxygen consumption values presented in Table-3

Weight of fish Gms.	15-25°C	25-30°C	30-35°C
1	1.86	1.54	5.95
4	2.09	1.44	2.37
10	2.38	1.25	1.32
16	2.47	1.25	0.97

The Q_{10} of oxygen consumption in *Puntius sophore* decreases with increasing temperature between 15 and 30°C but increases with increasing temperature between 25 and 35°C excepting in the 16 gm. fish (Table 4). The decrease in the Q_{10} values between 15 and 30°C is systematic and graded in smaller fish but becomes less and less so in larger fish and consequently in the 16 gm. fish the Q_{10} drops from 2.47 to 1.25 between 15-25°C and 25-30°C ranges, while in a 1 gm. fish the corresponding drop is only from 1.86 to 1.54. Conversely the increase in the Q_{10} values between 25-30°C and 30-35°C ranges is most marked in smaller fish but it becomes less and less so in larger ones. Thus in a 1 gm. fish the Q_{10} increases from 1.54 between 25 and 30°C to 5.95 between 30 and 35°C, while the corresponding increase in a 10 gm. fish is only from 1.25 to 1.32. This tendency appears to have proceeded much further in the 16 gm. fish, where the Q_{10} , instead of increasing, actually decreases from 1.25 between 25 and 30°C to 0.97 between 30 and 35°C. This negative Q_{10} value is a clear indication of marked heat depression in larger fish at 35°C.

DISCUSSION

That metabolism of animals is considerably influenced, among other factors, by body size and habitat temperature has long been recognised. In *Puntius sophore* the total oxygen consumption increases with the increasing body weight at all temperatures (Fig. 1). Conversely, the weight specific QO_2 decreases with increasing body weight. It has been pointed out that in a great majority of cases, studied amongst fishes, the increase in the oxygen consumption with size is surface area dependent rather than weight dependent (Bishop, 1950; Fry, 1957). Nevertheless there are cases like *Salvelinus fontinalis* (Job, 1955) and *Etroplus maculatus* (Parvatheswararao, 1959), where the increase in the oxygen consumption with size was found to be intermediate between surface area dependence and weight dependence, at all temperatures in the former and at the habitat temperature in the latter. As against this, in the present case the regression values at the habitat temperature and near about it (25° and 30°C) indicate that the increase in the oxygen consumption is nearly weight dependent rather than surface area dependent. But, that no generalisations can be made in this regard is shown by the fact that in the present case the increase in the oxygen consumption may follow surface area dependence (as at 35°C) or weight dependence (as at 25° and 30°C) or may even be intermediate to these two conditions (as at 15°C).

The fact that fish of different body weights have different response patterns to temperature variations is evidenced by the b values of the size-metabolism curves (Fig. 1). At 25°C (habitat temperature) the b value is maximal, being 0.96. At higher temperatures the b drops down to 0.91 at 30°C and 0.59 at 35°C (Table 2). That this is due to the smaller fish being more sensitive to temperature changes is obvious from the Q_{10} values shown in Table 4. At these higher temperatures the smaller fish have high Q_{10} values indicating greater increase in their metabolism with increasing temperature and consequently at higher temperatures the curve towards the lower weight ranges is elevated much more than the rest; hence decreasing slope of the curve with increasing temperature. At 35°C the low b value of the curve appears to be due to the larger fish being heat depressed at this temperature as can be made out by the negative Q_{10} value in the 16 gm. fish and also the smaller fish being extremely sensitive to but tolerant of this temperature as can be made out by the very high Q_{10} value in the 1 gm. fish at this temperature. Likewise when the temperature is lowered to 15°C from 25°C, the b value again decreases to 0.86 from 0.96 and in this case the decrease in the b value appears to be due to the larger fish being slightly cold depressed at 15°C, as can be seen from the high Q_{10} value of the 16 gm. fish at this low temperature. Thus in *Puntius sophore* the b value of the size-metabolism curves decreases with increasing temperature above the habitat temperature (25°C) as in the case of the fresh water fish, *Etroplus maculatus* (Parvatheswararao, 1959) and the earth worm, *Megascolex sp.* (Saroja, 1959). But unlike these instances cited, in *Puntius sophore* at 15°C the b value, instead of increasing, actually decreases, apparently due to the larger fish being cold depressed at this low temperature. Consequently, at the habitat temperature the b value happens to be maximal. Similarly, in the marine and brackish water populations of the prawn, *Metapenaeus monoceros* (Rao, 1958) the b values were maximal in the normal media and decrease with the variations in the salinity of the medium. This is especially true of the marine populations. In the lizards, *Sceloporus occidentalis* and *Uta stansburiana* the b values varied with temperature between 0.47 and 0.64 for the former and between 0.54 and 0.68 for the latter (Dawson and Bartholomew, 1956). But unlike what is found in the present case, the b value in the case of these lizards did not show any temperature trends. It appears that no general case can be made out for the b values as regards their temperature trends. These values are said to be variable even in the same species due to varying environmental conditions (Rao and Bullock, 1954; Rao, 1958).

It may be that the low b values in the present case at 35°C and 15°C are correlated with the heat depression at 35°C and slight cold depression at 15°C in the larger fish. Unlike this in *Etroplus maculatus* (Parvatheswararao, 1959), another fresh water fish, no heat depression was noted in larger specimens but indications of cold depression were noted in smaller specimens at 15°C. It may be reasonably assumed that the occurrence of heat depression in the larger specimens of *Puntius sophore* may be due to the fact that they were comparatively cold adapted, having been kept at 25°C, unlike *Etroplus maculatus* which were kept at 30°C. While the smaller specimens could tolerate this higher temperature extreme, the larger ones could not, consequent upon which their metabolism got depressed. The metabolic response to temperature changes in *Puntius sophore* is greater between either 25 and 35°C or 25 and 15°C than between 25 and 30°C, obviously due to the much wider gradient between the acclimated and experimental temperatures in the former than in the latter (Table 3 and Fig. 2). Similarly, in the crab, *Pachygrapsus crassipes* (Roberts, 1957) it has been shown that as between crabs acclimated to 8.5 and 16°C. the former consumed more oxygen at 23.5°C.

In metabolic studies the desirability of taking body weight into consideration can be well appreciated if the temperature responses in the metabolism of animals of different weight groups are to be understood relatively. In the present case

the Q_{10} of oxygen consumption is size dependent, although to varying degrees, being maximal at the highest temperature range (30–35°C) and less so at lower ranges (Table 4 and Fig. 3). This maximum size dependence of the Q_{10} values at 30–35°C appears to be exaggerated by the marked heat depression in the larger specimens and extreme sensitivity and tolerance of smaller specimens at 35°C. The reversal in the size dependence of the Q_{10} values at temperatures above and below the habitat temperature in the present case is of interest. Above 25°C the Q_{10} decreases with increasing body weight and below 25°C it increases with the increasing body weight. This appears to be a reflection of the manner in which the larger fish respond to changes in temperature, especially when the gradient is sufficiently large. Thus the largest fish has the highest Q_{10} at the lowest temperature and the lowest Q_{10} at the highest temperature, due to marked metabolic depression. Hence in this case the suggestion of Bělehrádek (1930, 1935) that physiological activities, provided they are not of a low coefficient, generally show an increasing temperature sensitivity with age appears to be true to a certain extent. It has been pointed out (Rao and Bullock, 1954) that temperature coefficients in poikilotherms most commonly either increase with increasing body weight or show no size trend at all. And both these trends were found in the crab, *Pachygrapsus crassipes* (Roberts, 1957) but at different temperature ranges, the Q_{10} increases with increasing body weight between 16 and 23.5°C and shows no size dependence between 8 and 16°C.

In *Puntius sophore* the Q_{10} does not seem to show any regular and systematic variation with temperature excepting in the 16 gm. fish (Table 4). Between 15 and 30°C it decreases with increasing temperature but between 30 and 35°C it increases except in the 16 gm. fish, in which due to acute heat depression at 35°C the Q_{10} drops to less than one. Unlike this, in another fresh water fish, *Etroplus maculatus* (Parvatheswararao, 1959) the Q_{10} systematically decrease with increasing temperature. In the earth worm, *Megascolex* sp. (Saroja, 1959) also the Q_{10} values did not show any regular temperature trend. However, in the present case, it may be noted, that the Q_{10} values are less size dependent at lower temperatures than at the higher and the greater size dependence of the Q_{10} values at higher temperatures appears to be further exaggerated by the marked heat depression in the larger fish at 35°C. As against this in *Etroplus maculatus* there is a greater size dependence of the Q_{10} values at a lower temperature range like 15–30°C, perhaps due to the cold depression of smaller fish at 15°C (Parvatheswararao, 1959). In *Mytilus californianus* (Rao, 1953) also, as in the present case, the Q_{10} values were found to be less size dependent at lower temperatures.

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STUDIES ON PALMS : PART IV—ANATOMY OF PALM ROOTS

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ABSTRACT

The paper gives an account of the structure of adult root in 37 species of palms representing all important subtribes of the Palmae except the Mauritiace of which the material was not available. The relative importance of anatomical characters of these species has been emphasized and a discussion as to their value in the diagnosis of species on anatomical basis has been given. It has been suggested that hypodermis, air cavities and fibre bundles in the cortex which are of three types: *Phoenix*, *Arecu* and *Hyophorbe*, nests of sclereids on endodermis, characters and nature of stele, monostelic or polystelic, its configuration due to lignification of the conjunctive parenchyma, thickened, or unthickened central pith, presence or absence of medullary bundles in it, are features highly characteristic in each species and can be depended on for the purposes of analysis of palm species on the basis of their anatomy. This aspect of the work has been fully discussed on pp. 92-95. It has been further suggested that since many of these characters are of a stable nature, they can be used for the analysis of the artificial genus of palm roots, "*Rhizopalmozylon*".

I. INTRODUCTION

Palms form characteristic vegetation of coast line throughout tropics and oceanic islands. They are an ancient family ranging from Triassic to Modern period, a number of them having had flourished in the Tertiary Flora of many lands. Most of the living palms are concentrated in the Indo-Malayan region in the Old World and in the tropical islands of the New World. Studies on them are fascinating as they have woody, arborescent habit, primitive floral characters, economic importance, besides their availability as fossils in the Tertiary horizons all over the world. Solereder and Meyer (1928) have summarised work on the anatomy of living palms till 1928 dealing with stem, leaf, epidermis and roots, while a review of literature on fossil palms till 1943 has been given by Stockmans and Williere (1943), and by Mahabale (1958). Stray references to their anatomy as monocotyledons are also available in Arber (1922), Cheadle (1941, 1942, 1943a, 1943b, 1944, 1953), Bailey (1944), and others, but there is no connected account of the group as a whole. The senior author and his associates have also been working on palms for the last 12 years and the main findings have been published from time to time (Mahabale and Udwadia, 1953; Mahabale and Chennaveeriah, 1953, and Mahabale, 1958). Much of this work deals with morphology, anatomy of vegetative and floral parts, and cytology. The present paper deals with the anatomy of roots in 35 species of living palms belonging to 10 genera, 20 of which have not been previously described. They are, therefore, described in detail here.

Roots in palms possess some peculiar features such as the so-called polystele due to dissolution of stelar system into strands of various shapes, changes in their anatomy due to varied habitats, etc. Some of these features are characteristic of certain genera and species and they help in identifying them on the basis of their anatomy. Anatomy of roots in living palms has been described by Von Mohl (1849), Gillain (1890), Cormack (1896), Drabble (1904), Schoute (1912) and Bower (1923), whereas, Stenzel (1904), Sahni (1938), Gothan (1942), Stockmans and Williere (1943), Shukla (1946) and Ogura (1952) have described it in a few fossil species.

II. MATERIAL AND METHODS

The roots of palms generally arise from the basal part of a tree trunk where they form a dense net-work surrounding it. They are adventitious in origin and pierce their way from below the bark and enter soil. Some of them are long and reach a distance of about 40' as was observed by Yampolsky (1924) in *Elaeis guineensis*. Some of them become thick and mechanical in function and run obliquely and serve as prop or stilt roots as those in *Rhizophora* or *Pandanus*. Others are small and grow downwards and serve as absorbing organs. The prop roots also on reaching soil send out smaller roots. In some palms the secondary roots grow vertically upwards and form *pneumatophores* e.g., in *Versaffeltia splendida*, *Phoenix sylvestris*, *Phoenix humilis*, *Elaeis guineensis*, etc. Others do not enter soil at all, but form an aerial jacket around the tree trunk at one or two places. The origin and gross structure of such roots has been discussed by Von Mohl (1849) and later by Cormack (1896) and Drabble (1904), but in the present paper only the structure of adult aerial and underground roots in transverse section has been given without entering into discussion as to their origin.

As a general rule, anatomically the roots of palms are highly complex at the point of their origin from a stem, but get simplified later towards the growing point as they emerge from the cortex of stem and enter soil. A growing root, therefore, when sectioned in series at different lengths from its apex back to its origin in the stem cortex, it presents different appearance at different places, besides the changes it shows in adaptation to its habitat, and thus a good deal of variation in structure is found in palm roots, sometimes even in the same species.

In the present work both wild and cultivated species were investigated. The roots soon after collection were cut into 10-15 micra thick sections with the help of a Leitz's Table Microtome, as the fixed material in palms gives rather unsatisfactory results. For studying anatomical structure, each root was sectional in proximal, middle and distal portions two to three inches away from the point of its origin. In many palms distal and middle parts of a root remain underground, but the proximal part is exposed to air. Both young and adult roots were sectioned, but the sections of only middle part of adult roots were selected here for comparative study. Sections so cut were fixed in formalin-acetic-alcohol (Alcohol 95 per cent 90 c.c., Commercial formalin. 5 c.c., Glacial acetic acid, 5 c.c.) for 2-3 days, washed and stained with alcoholic safranin and light green dissolved in clove oil. After dehydrating they were cleared in xylol and mounted in semi-fluid Canada balsam till dried in an oven, adding a little weight on slides for uniform drying. They were sketched with the aid of Zeiss Camera lucida at the level of the stage at magnification indicated separately against each figure.

III. KINDS OF PALM ROOTS

All palm roots as stated above arise adventitiously from stem. For the purposes of understanding they may be classified as follows :—
(1) *Large-sized aerial roots functioning as prop roots :*

These are stout and plagiotropic and extend over a long distance. They produce secondary or tertiary roots in soil later, e.g., in *Areca catechu*, *Elaeis guineensis*, etc.

(2) *Cluster-forming aerial roots* :

These arise from below the rind of trunk, in basal or epibasal region, a little above the ground and form a thick jacket of roots around the tree trunk once, rarely twice as in *Hyphaene indica*. They grow downwards at various angles.

(3) *Pneumatophores* :

Some roots arising from the above two categories, and in some species those arising from below the tree trunk, very close to the ground, produce *negatively* geotropic roots which function as pneumatophores, e.g., in *Versaffeltia splendida*, *Phoenix paludosa*, *Phoenix sylvestris*, *Elaeis guineensis*, etc. In *Versaffeltia splendida* they look like the stilt roots of mangroves but in *Phoenix* species they are small and grow near the surface of soil and look like coralloid roots of *Cycas*.

Gillain (1890), Haberlandt (1914), Yampolsky (1924), d'Almeida and Correa (1949) consider them to be true pneumatophores, but this view is not shared by all. They occur in the above palms which are dwellers of xerophytic habitats. Presumably the ancestors of these palms were growing on coastal streams in the past and migrated to their xeric habitats later, and, therefore, the habit of forming pneumatophores is lingering in them.

(4) *Normal absorbing roots* :

These arise as secondary or tertiary roots on the underground parts of any of the above three kinds. They are comparable with the absorbing roots of other plants.

Anatomically all these roots differ from one another. A normal absorbing underground root has a thin limiting layer, epiblema, generally without root hairs. It has 2-3-layered hypodermis, the middle layer being thin. The cortex is large and parenchymatous with or without fibre bundles (cf. Figs. 5, 10, 13 and 26). In the middle part of cortex, air spaces of different size and shape are formed lysigenously, except in *Nipa* where they are formed schizogenously. The inner cortex is made up of small cells abutting on endodermis, sclereids, and some melanogenic cells. The outer cortex contains many rephide sacs lined by epithelial cells of parenchyma. Endodermis is generally one-layered, but in negatively geotropic pneumatophores, it may be two-layered. Pericycle is single-layered. 20-30 xylem and as many phloem strands alternate with each other and lie radially below it. The conjunctive parenchyma is highly lignified, but the pith in the centre is generally unthickened. The outer fringe of central pith is thrown into folds of various shape. Medullary bundles occur in it sometimes.

The large-sized aerial roots and prop roots retain essential anatomical features of normal absorbing roots, namely, lacunar cortex, thick-walled conjunctive parenchyma of the stele, thin-walled central pith, medullary bundles, cortical fibre strands, etc. but the air spaces in middle cortex increase in size, especially in their underground parts, and the limiting layer and hypodermis are not heavily thickened. The secondary and tertiary roots have compact tissues. Conjunctive parenchyma in them is strongly developed and is thick-walled. The central pith is many-layered and extensive, partly or fully thickened.

In *pneumatophores* arising from large-sized secondary aerial or underground roots, epiblema or the limiting layer, is thick-walled and lenticular. Hypodermis is 10-12-layered, but is not thick-walled. Cortex is non-lacunar and is full of fibre strands. The stele has a smaller number of xylem and phloem strands, about 20, arranged radially. The conjunctive parenchyma completely fills the central cavity. The central pith is unthickened in them as in ordinary absorbing roots.

Anatomically thus, barring little lenticular openings on them, described as *pneumathodes* by Haberlandt (1914), and their negative geotropic growth, there is little to suggest that they are different than true roots. Presumably they are not exactly the same as the pneumatophores of mangroves or the coralloid roots of *Cycas*, with which they resemble in appearance. Some of them even develop chlorophyll in outer and middle layers of cortex below hypodermis, possibly on being exposed to sunlight as do the aerial roots of *Vitis* or *Tinospora* or the pneumatophores of *Avicennia*.

Keeping these general characters of palm roots in mind, we shall now describe the structure of roots in different species and discuss points of general interest emerging from them at the end later.

IV. DESCRIPTIVE

The family *Palmae* is divided into five tribes and seven sub-tribes by Drude (1887) of which *Coryphineae* is the first. It is further sub-divided into *Phoenixeae* and *Sabaleae*. From the sub-tribe *Phoenixeae* five species of *Phoenix* were studied. Of these *Phoenix sylvestris* has been described as a type for all palm roots and differences shown by other species, viz., *Phoenix zeylanica*, *Phoenix rupicola*, *Phoenix paludosa* and *Phoenix dactylifera* are indicated briefly.

Phoenix sylvestris Roxb.

This common Wild Date Palm of India grows on moist ground throughout dry districts along the banks of streams and water courses and in the hills upto about 6,000'. Soon after germination, the primary root perishes and is replaced by adventitious roots arising from the stem. There are three types of root in it as reported by d'Almeida and Correa (1949): (i) The stout adventitious roots arising from the basal portion of the trunk growing obliquely in soil; (ii) Negatively geotropic ones growing as tough laterals given out by the stout adventitious roots near ground, and (iii) thin, branched roots also given out by stout adventitious roots forming a jacket around the trunk. These have no special direction of growth.

A transverse section of aerial portion of adventitious root, 2.7 m.ms. in diameter, has epiblema with large cells and dark contents. Epiblema is cuticularised, or lignified and is without root hairs (Fig. 1). The epiblema has been termed by different authors as epidermis, periderm, rhizodermis, limiting layer or piliferous layer. Drabble (1904) feels that term 'Limiting layer' is better since epidermis in palm roots or the piliferous layer, as a rule, has no hairs. On the other hand, Haberlandt (1914) prefers calling it as 'periderm'. In the present study, the term limiting layer or epiblema is used in a non-committal sense.

The epiblema often shows small warts and cracks. The hypodermis here is replaced by a mass of thin-walled spongy tissue which acts as pneumathodes according to Haberlandt (1914) and is common to many other palms also.

Below epiblema lies lignified hypodermis, with a strip of thin-walled parenchyma and two layers of lignified hypodermis. According to Drabble (1904), the intervening layer of thin-walled parenchyma is due to centrifugal thickening of cells in the outer and inner zones of hypodermis, leaving a strip of unthickened cells in the middle (Fig. 22, mh). The outermost thickened layers here form the tegmentary system of root (Drabble, 1904).

Below hypodermis lie the large cortex of thin-walled round cells with fibre bundles (Figs. 2 and 4). As a rule it has three regions: the outer, middle and inner. A fibre bundle in the cortex is circular, surrounded by silica crystals, the stegmata, arranged in discontinuous longitudinal rows. It forms a type of fibre bundle by itself, the *Phoenix*-type, called as *Raphia*-type by Drabble (1904) so as to include genera *Raphia*, *Pinanga*, *Phoenix*, *Metroxylon*,* *Dictyospermum* and *Seaforthia* under it. The fibre bundles in the following additional palms may also be classified

under the *Phoenix* type, namely, *Corypha*, *Hyphaene*, *Caryota* and *Martinezia* (see Figs. 13, 28, 34 and 66). They probably secure extensibility of main roots anchoring plant to the ground.

Raphide sacs containing acicular crystals and a few tannin and mucilage cells are also present in the middle cortex.

The central vascular cylinder is so constructed as to ensure pliancy combined with tensile strength. The polyarch exarch stele has 17 arches of xylem and phloem.

The lignified endodermis is one-layered and belongs to Russow's "C" type described by Haberlandt (1914). Exodermis is not much thickened whenever present. Pericycle is one-layered but at places two-layered. Xylem and phloem lie radially in thin-walled conjunctive tissue, the rest of it being lignified. The central pith is small about 75μ .

The secondary roots arising from the large pull roots are circular, about 4.120 mms. in diameter (Figs. 2, 3). They differ from the aerial roots described above in having distinctly lacunar cortex, full of lysigenously formed air cavities. In larger roots these air spaces enlarge further by disintegration of cells, always noticeable at their boundaries. Remains of some partially dissolved cells are seen in *Kentia*, *Metroxylon rumphii* and *Phytelephas macrocarpa*.

An incomplete ring of pigmented cells, containing tannin, is seen in the inner cortex. This was not observed by Drabble (1904). The stele here consists of 36 arches of xylem and phloem. The protoxylem cells get thickened before the metaxylem (see Cheadle, 1943a). The vessels are spread centrifugally. The large scattered vessels appear first, followed by smaller metaxylem cells and protoxylem. According to Buscalioni (1902) and Pirotta (1902), as cited by Drabble (1904), this is so in several other monocotyledons also.

The phloem strands are slightly broader towards the central pith and taper towards pericycle. They lie in a single series alternating with xylem strands. The endodermis and pericycle are similar to those in larger roots. Generally one medullary bundle of hydrocentric type is present in the central pith. It consists of a metaxylem cell surrounded by phloem ring and lignified conjunctive tissue. Several such medullary bundles have been reported in the central pith of root in this species by d'Almeida and Correa (1949), though Drabble (1904) had not seen them in this species.

Phoenix zeylanica Trimen

This date palm is common in moist places in Ceylon.

A transverse section of aerial portion of an adventitious root is circular, about 7.680 mms. in diameter in the middle part (Fig. 4). The outer-most limiting layer has no hairs. Its cells are lignified and have dark contents as in *P. sylvestris*. Below it lies lignified hypodermis followed by a large cortex with thin-walled rounded cells and fibre bundles of the *Phoenix*-type.

The air-spaces in the middle cortex lie in 2-3 rows. Raphide sacs, tannin and mucilage cells are also present as in *P. sylvestris*. The ring of pigment cells in the inner cortex is incomplete. The endodermis and pericycle are similar to those in *P. sylvestris*.

The stele consists of 47 arches of xylem and phloem radially placed in thin-walled conjunctive parenchyma. The remaining part of conjunctive parenchyma is lignified. The phloem strand is elongated, slender and conical. It is narrower and longer than *P. sylvestris*.

The central pith, 1.040 mm. wide, is made up of thin-walled, round cells with three medullary bundles. Each medullary bundle has a large xylem vessel in the centre, and a few protoxylem vessels along the large ones. Drabble (1904) and Haberlandt (1914) had not seen them in their material.

The exact nature and function of the medullary bundles is controversial. Drabble (1904) is of opinion that some of the more internal procambial strands which do not enter into the fibrous ring of conjunctive parenchyma during development, persist only as medullary strands, each presenting proximally a radial structure with xylem and phloem developed well but reduced distally. Thus we find : (1) strands containing only a few xylem vessels accompanied by one or more phloem groups, or (2) one or two large vessels with a few smaller ones unaccompanied by phloem, or (3) a single large xylem vessel without a smaller vessel or phloem. The last mentioned two types are more common in *Phoenix*.

Haberlandt (1914) is of opinion that the medullary bundles occur as accessory strands within the primary central vascular cylinder of a root and serve in the first instance in transportation of material to the whole length of that organ, at any rate over a considerable distance of it.

Phoenix rupicola T. Anders.

The slender stem of this most handsome species has no girdle of aerial roots as in two previous species. It grows on rocks in Sikkim Himalayas, Assam and Mishmi Hills.

A transverse section of an underground adventitious root, about 7.120 mms. in diameter, has epiblema and hypodermis as in *P. sylvestris* (Fig. 5). The cortex consists of thin-walled round cells with fibre bundles of the *Phoenix* type. Air cavities in the middle cortex are arranged in 2-3 rows. Raphide sacs, tannin and mucilage cells are also present in the cortex. The ring of pigment cells in the inner cortex is incomplete. The circular stele has 43 arches of xylem and phloem. Endodermis and pericycle are as in *P. sylvestris*. The xylem strands are "V"-shaped, metaxylem being embedded in the lignified conjunctive parenchyma. The central pith, about 960 μ , consists of thin-walled round cells with a few air cavities.

Phoenix paludosa Roxb.

This sub-arboreous, gregarious palm grows on estuarial shores from Bengal to Burma, and in Andaman Islands. It forms a considerable portion of impenetrable woods in Sunderbuns, Amherst, Moulmein, Penang, Siam and Cochin-China.

A transverse section of an underground adventitious root, about 8.480 mms. in diameter, has epiblema and hypodermis as in *P. sylvestris*, but hypodermis is comparatively narrow (Figs. 6-7).

The outer cortex consists of thin-walled round cells with little intercellular spaces. Middle cortex is made up of highly elongated cells, 40 μ \times 28 μ , not seen in other species. Air cavities in the middle cortex are very large, due to its halophytic habit. Fibre bundles are of *Phoenix* type. Raphide sacs, mucilage cells and tannin cells are present in the cortex. The stele is circular and consists of 45 arches of xylem and phloem. Endodermis and pericycle are as in *P. sylvestris*. The xylem and phloem strands are radially disposed, partly in thin-walled conjunctive parenchyma. Rest of the conjunctive parenchyma is lignified. The central pith, about 1200 μ , is slightly lacunar; and three medullary bundles with metaxylem cavities (Fig. 12) are present in it.

Phoenix dactylifera L.

This edible date-palm has a large trunk, often surrounded by a dense mass of root-suckers, a foot above the ground. It is cultivated or self-sown in Sind and Southern Punjab and thrives best in arid, rainless regions of North Africa and West Asia.

A transverse section of the aerial portion of an adventitious root, 6-80 mms. in diameter, has epiblema with dark contents (Fig. 8). The limiting layer is broken

at places by pneumathodes. Below it is seen, as in *P. sylvestris*, cortex with air cavities, fibre bundles of the *Phoenix* type, raphide sacs, tannin cells and mucilage cells. An incomplete ring of pigment cells is present in the inner cortex. The stele has 25 arches of xylem and phloem radially disposed separated from each other by conjunctive parenchyma completely sclerosed nearly to the centre of the section (Fig. 8). A few passage cells are seen opposite the protoxylem groups in endodermis. According to Drabble (1904) no such passage cells occur in the underground region of the roots in this species. Occasionally xylem strands are "V"-shaped; a few are "I"-shaped. Phloem strands are generally large, but when small, they lie between the arms of "V"-shaped xylem. The pith, 240μ wide, consists of thin-walled round cells. No vessels getting abstricted in the pith as those seen by Cormack (1904) and Haberlandt (1914) were noticed here.

Chamaerops humilis L. Hort.

This Dwarf Fan Palm varying 5'-15' in height, is the only palm indigenous to Europe and grows in countries bordering the Mediterranean Sea.

A transverse section of underground portion of the root, about 4.6-5.0 mms. in diameter, has epiblemma of thick-walled cells with dark contents (Fig. 9). Below it lies large hypodermis of lignified cells and cortex of thin-walled round cells. Air-spaces in the middle cortex are arranged in 3-4 rows. Raphide sacs, tannin cells and mucilage cells are present in the cortex. An incomplete ring of stone cells and pigment cells occurs on the inner cortex. Endodermis is continuous, lignified and of "C"-type. Passage cells lie over the protoxylem elements. Pericycle is one-layered. The stele has 36 arches of "I"- "V" or "Y"-shaped, xylem, phloem lying in their midst. The phloem strands are oval and elongated, their broad side lying towards the centre. The conjunctive tissue is thin-walled towards periphery but lignified towards the central pith, 240μ , with round cells without air-cavities.

Trachycarpus muritiana H. Wendl.

This tall slender palm of Central Himalayas, Eastern Kumaon and Upper Burma has a network of underground adventitious roots.

A T.S. of old underground root measuring 6.960 mms. in diameter has a limiting layer as in the previous species (Fig. 10). There is a broad band of hypodermis having three distinct zones. The cortex consists of oval or round thin-walled cells and small triangular intercellular spaces. It also includes mucilage cells and numerous raphide sacs. A complete wavy sheath of sclerenchyma surrounds endodermis of the "C"-type. One-layered pericycle is lignified over the phloem strands but is unligified over the protoxylem strands. The stele is 35-arched. Xylem and phloem strands lie radially, separated by sclerosed conjunctive tissue. The xylem groups are "I"- or "V"-shaped, with phloem strands lying in between them. The innermost cells of the xylem strands, sometimes separated by a row of lignified parenchyma, are very large and they form a conspicuous row of cells below the regular metaxylem cells. Central pith measures 640μ , its cells being thin-walled and round. Air cavities, mucilage cells and tannin cells occur in it.

Rhapis flabelliformis Ait-Hort.

This Dwarf Ground Rattan, with stem 5'-6' high and as thick as thumb, is ensheathed by reticulate persistent bases of oldern leaves. It is native of South China and Loochoo and is cultivated in many parts of India and Europe.

A T.S. of the underground root is about 3.290 m.ms. in diameter. Its limiting layer and hypodermis are lignified. A few raphide sacs and mucilage cells are present (Figs. 11, 12). The cortex has oval or round, thin-walled cells with inter-

cellular spaces. The air cavities are arranged in 1-2 rows. Endodermis is of 'C'-type, and pericycle one-layered. The stele consists of 55 arches, xylem and phloem lying on separate radii. A few large metaxylem cells lie apart from protoxylem centripetally. Whole of the vascular tissue lies in a sclerotic ring of conjunctive tissue and is zig-zag in outline internally. Xylem strands are 'I', 'V' or 'Y'-shaped, phloem lying in their midst. The central part of the root is occupied by unlignified pith, 1.280μ , with raphide sacs, and 8-9 medullary bundles having metaxylem cavities (Fig. 12) : a few of them even touch the sclerotic ring of conjunctive parenchyma. The central pith has no air cavities.

Corypha umbraculifera L.

This Talipot Palm 8'-16' high with large palmate leaves is common in Ceylon and Malabar coast. According to Drabble (1904), there are two types of roots in this species : (1) Large contractile roots with transverse wrinkles on outer surface of the whole root and (2) non-contractile roots. Both these roots were examined, but they showed very little variation in their internal structure. The present study describes non-contractile roots.

A T.S. of an underground non-contractile adventitious root, about 11.680 mms. in diameter, has 40μ epiblemma made up of lignified cells with dark contents (Figs. 13-20). Large hypodermis of lignified cells adds to the mechanical strength. Air cavities in the cortex are arranged in 7-8 rows, and fibre bundles of *Phoenix* type occur in the cortex (Figs. 13-19). Raphide sacs (Fig. 19), tannin cells and mucilage cells are also present. There is an incomplete ring of pigment cells in the inner cortex (Fig. 20). The central vascular cylinder is highly elaborate, consisting of one-layered endodermis with Russow's 'C'-type of thickening (Fig. 20). Pericycle is one-layered. The stele consists of 55 arches. Xylem and phloem are embedded in the conjunctive tissue which is thin-walled towards the outer side and lignified partly below (Fig. 20). Xylem strands are I-shaped. Phloem strands are solitary, or at places split into two longitudinally or transversely, and are bridged by conjunctive sclerenchyma (Fig. 13). The pith, 5.760 mms. in diameter consists of fairly large cells, $160 \times 96\mu$, with compact 70 medullary bundles of different kinds. Of these, 18 medullary bundles were leptocentric (Fig. 18), their phloem having been surrounded by conjunctive tissue. A few of the phloem bundles were fused (Fig. 16). 46 medullary bundles were hydrocentric (Fig. 14), each with a xylem strand in the centre surrounded by a ring of phloem and conjunctive tissue outside. A few bundles of this type also were fused (Fig. 15). The remaining 6 medullary bundles were compound; the leptocentric and hydrocentric bundles having been fused together (Fig. 17). Besides these bundles fibre bundles of *Areca* type are present in the pith (Fig. 13). The fibres were generally collected in masses of irregular shape. They were short. Their walls were thin and lumen large. Drabble (1904) noticed the same in *Kentia*, *Areca*, and *Cyrtos-tachys*. In addition to these *Chrysalidocarpus lutescens*, *Howea belmoreana* and *Archontophoenix cunninghamii* also have *Areca*-type of fibres. Air cavities were present in the pith. Drabble (1904) who had described this species had not noticed different kinds of medullary bundles or the fibre bundles.

Licuala peltata Roxb.

The lower portion of this gregariously growing palm is marked with the scars of fallen leaves. It is common in Sikkim, Assam, Burma, Andaman Islands and is cultivated in gardens.

A T.S. of an underground root is about 3.808 mms. in diameter, with a limiting layer having thick-walled tabular cells as those in exodermis (Figs. 21, 22). Hypodermis is broad consisting of three zones. The outer and inner zones have lignified

cells but the middle one is thin-walled (Fig. 22). The cortex has two types of cells: oval, round, thin-walled cells, $48 \times 43\mu$, and thin-walled, elongated cells, $160 \times 100\mu$. Air cavities are arranged in 2-3 rows. They are elliptical and lysigenous formed. An incomplete ring of sclereids and pigment cells are present on the inner cortex (Fig. 31). Several raphide sacs, mucilage and tannin cells occur in the cortex. Endodermal cells are of "C"-type. Pericycle is highly conspicuous being interrupted by thin-walled cells near protoxylem strands. 37 arches of xylem and phloem "I"- or "V"-shaped lie radially in a sclerotic ring of conjunctive tissue. The central pith, $400 \times 800\mu$ makes several incursions into the conjunctive parenchyma, and has a single medullary bundle of hydrocentric type.

Licuala grandis H. Wendl.

This palm of New Britain is introduced in Indian gardens.

An underground root of this palm is 5.360 mms. in diameter in the middle. Its epiblemma is similar to that of *L. peltata* (Fig. 23). Hypodermis is lignified and has two equal parts: the outer with dark contents and inner without them. The cortex is made up of oval, thin-walled cells, and 2-3 rows of air-cavities. An incomplete ring of sclereids and pigment cells are present in the inner cortex. Raphide sacs, mucilage cells and tannin cells occur throughout the cortex. The stele consists of about 36 arches of xylem and phloem. The endodermal cells are of "C"-type without any passage cells. Pericycle is completely lignified. Xylem and phloem strands lie on different radii in the sclerosed conjunctive tissue. Large metaxylem cells lie in its ridges with no thin-walled parenchyma around them. In thicker roots phloem strands are like those in *C. umbraculifera* and are arranged in two series with large and small phloem strands. The central pith is 1.792 mms. and has 7 or 8 medullary bundles each having metaxylem cavities. A few raphide sacs, mucilage cells and air cavities are also present in the pith. Some of the medullary bundles lie close to the ring of conjunctive parenchyma and form compound medullary bundles.

Livistona chinensis T. Br.

This *Livistona* species common in China and Japan is cultivated in India.

A transverse section of an underground root, about 8.800 mms. in diameter, has a thick-walled limiting layer (Figs. 24, 25), often exfoliated (Drabble, 1904). Hypodermis consists of three zones as in *P. sylvestris* and *L. peltata*. The cortex consists of thin-walled round cells traversed by 2-3 rows of spindle-shaped lysigenous air cavities. A few raphide sacs, mucilage and tannin cells are present in the cortex. In older roots, slightly thicker, parenchymatous cells get lignified here and there. An incomplete ring of sclereids and pigment cells are present in the inner cortex. The endodermis and pericycle are as in the last species. About 56 arches of "I"- or "V"-shaped xylem alternate with phloem in lignified conjunctive tissue. The "V"-shaped xylem strands have large metaxylem cells towards pith, their arms extending towards the pericycle. Phloem is generally included in the form of "V" of xylem or may remain as a distinct strand if xylem is "I"-shaped. The central pith 2.080 mm. in diameter, consists of thin-walled round cells and has two medullary bundles of hydrocentric type unlike those in *L. grandis*. Air cavities and sclereids are also present in the central pith. Drabble (1904) has described this root but did not notice the presence of sclereids in the cortex and pith.

Sabal adansonii Guers.

This Dwarf stemless *Sabal* occurs in low humid forests and in inundated areas on sea-shore and sandy soil in south eastern part of the United States.

A transverse section of adult underground root, about 6.080 mms. in diameter, has epiblema, hypodermis and cortex as in *L. grandis* excepting that the cortical air-cavities are arranged in 3-4 rows (Fig. 26). There is a single incomplete ring of pigment cells in the inner cortex. Raphide sacs, tannin cells and mucilage cells are as in the last species. Endodermis is one-layered, with "C"-type of thickening. Pericycle is also one-layered. The stele consists of 33 arches of xylem and phloem. Xylem strands are "I"- or "V"-shaped, but the phloem strands lie in one series united at places by their lower ends to form "M"-shaped structure enclosing a small xylem strand between their arms. The presence of "U"-shaped phloem strand, fibre bundles of *Areca*-type and medullary bundles have not been mentioned by Drabble (1904) who had described it before. The phloem strands lie in one series. The central pith $640 \times 512\mu$ consists of thin-walled round cells. A few fibre bundles of *Areca*-type and 1-2 medullary bundles with metaxylem cavities are present in the central pith. At places the fibre bundles are fused with medullary bundles. Air cavities are also present in the central pith.

Sabal serrulata Roem. et Schultz

A transverse section of an adult underground root of this cultivated species is 7.040 mms. in diameter. Epiblema, hypodermis and cortex are similar to those in *Sabal adansonii*. The air cavities, however, are arranged in 2-3 rows in the cortex (Fig. 27). Raphide sacs, mucilage and tannin cells are present in the cortex. Cells in the inner cortex are thin-walled and transversely elongated. There is an incomplete ring of pigment cells in the inner cortex. The central vascular cylinder and pith are similar to those in the last species, the stele having 23 arches of xylem and phloem lying radially in thin-walled conjunctive parenchyma. The central pith, 480μ , consists of thin-walled round cells and has a few sclereids here and there.

Hyphaene indica Becc.

A transverse section of an adult underground root of this dichotomously branched Indian Doum Palm is about 9.600 m.ms. in diameter. Epiblema is lignified and has dark contents (Fig. 28). Hypodermis is also lignified. The cortex consists of thin-walled round cells with 1-2 rounds of air cavities in the middle cortex. Fibre bundles of *Phoenix* type are present throughout the cortex. An incomplete ring of pigment cells is present in the inner cortex. The raphide sacs, tannin and mucilage cells are similar to those in the above species. The stele consists of 41 arches of xylem and phloem radially disposed in the conjunctive parenchyma which is thin-walled towards the periphery and lignified towards the central pith. Xylem strands are "I"- or "V"-shaped. The large phloem strands about $80 \times 40\mu$, lie beside the "I"-shaped xylem strands. Small phloem strands, about 48μ , lie in between the arms of "V"-shaped strands. At places phloem strands are arranged in two series as in *C. umbraculifera*. The central pith, about 880μ , is without medullary bundles and air cavities.

Latania verschoffeltii Lemaire.

This palm abundant in Rodrigues Island is cultivated in India.

A T. S. in the proximal part of an underground root is about 9.960 m.ms. in diameter and has epiblema with dark contents. Hypodermis is lignified (Fig. 29). Air cavities in the cortex are arranged in one row in the middle as in *H. indica*, but a large number of sclereids are present throughout the cortex. An incomplete ring of sclereids, and pigment cells are present in the inner cortex. The stele consists of 37 arches of xylem and phloem lying radially in the conjunctive parenchyma. Endodermis is of the "C"-type and pericycle is one-layered. Xylem

strands are "I"- or "V"-shaped. Large phloem strands lie by the side of "I"-shaped xylem, smaller strands lying in the arms of "V"-shaped xylem. The central pith, about 600μ , consists of thin-walled round cells and has sclereids scattered in it.

A T.S. of the distal part of a root about 10.880 m.ms. in diameter has epiblema, hypodermis and cortex as in the last species but air cavities are arranged in 2-3 rows (Fig. 30). Raphide sacs, tannin and mucilage cells and sclereids are also present. The sclereids form a complete ring in the inner cortex. The stele consists of 43 arches of xylem and phloem embedded in the conjunctive tissue, but here and there phloem strands are "U"-shaped. The xylem strands have metaxylem extended more towards central pith and are "Y"-shaped. The central pith, about 1040μ , consists of thin-walled round cells without any medullary bundles. A few air-cavities are present in it.

Borassus flabellifer Linn.

The roots of Palmyra palm are very long, some times 50' or more. Portion of trunk near the ground has a dense mass of long rootlets.

A T.S. of an aerial adventitious root, about 7.360 m.ms. in diameter, has a thick-walled lignified layer, below which lies a thin-band of hypodermis made up of polygonal lignified cells, filled with a dark brown substance (Fig. 31). The cortex is made up of oval or round, thin-walled cells with small triangular inter-cellular spaces. Air cavities in the middle cortex are irregularly arranged in 1-2 rows. Many sclereids, raphide sacs, mucilage cells and tannin cells occur in the outer and inner cortex. A complete ring of sclereids and an incomplete ring of pigment cells are present in the inner cortex. The stele consists of 47 arches of xylem and phloem. Pericycle is double and the radial walls of endodermis are thickened due to Casparian bands. Below pericycle is a distinct band of thin-walled conjunctive parenchyma in which xylem and phloem strands lie radially immediately below pericycle. Another row of phloem strands alternating with large metaxylem cells follows them, and then there is a zone of lignified conjunctive parenchyma, zig-zag in outline towards the central pith. It includes a few large xylem strands which appear to be abstricted in the pith later. The central part of root about 1200μ , is occupied by an irregular patch of parenchyma enclosing one or two medullary bundles having metaxylem cavity. A few air cavities are present in the central pith.

Calamus tenuis Roxb.

This climbing species of Tropical Himalayas, Bengal, Assam, Burma, and Cochin-China, has very long roots. A T.S. of an underground root is about 5520 m.ms. in diameter (Figs. 32). The cells of epiblema are large and radially arranged, their walls being highly cuticularised. Hypodermis is made up of lignified cells of which the outer 7-8 layers are very compact and are filled with a dark substance. The inner lignified cells are empty. The outer cortex has a few raphide sacs; the middle cortex has air cavities lying in 1-2 rows. An incomplete ring of pigment cells is present in the inner cortex. Raphide sacs, mucilage and tannin cells lie scattered throughout the cortex. Endodermal cells are radially elongated and are thickened on all sides. They conform to the "O"-type of endodermal cells of Russow. Pericycle is two-layered. The stele consists of 42 arches of xylem and phloem radially arranged, the former being "I"-shaped or "V"-shaped. Phloem is generally included in the fork of "V" or may lie outside. The apex of "V" is formed by a very large metaxylem cell lying in the sclerosed conjunctive tissue. Some of the cavities of the large metaxylem cells are either completely blocked or partially obliterated by bladder-shaped intrusions, the tyloses. The central pith is large, 1680μ , and has thin-walled rounded

cells enclosing 6-7 medullary bundles each with a metaxylem cavity only. Fused medullary bundles, a few raphide sacs and tannin cells are also seen in the central pith.

Calamus ratang L.

A T.S. of an underground root of this Indian cane palm is about 5,520 m.ms. in diameter. Epiblema, hypodermis and cortex are similar to those in the last species, except that air cavities in the middle cortex are large (Fig. 33). Raphide sacs, mucilage and tannin cells and an incomplete ring of pigment cells are present as in this species also. The stele has 37 arches of xylem and phloem. The vascular cylinder is similar to that in the last species. The central pith is 960μ . It is without medullary bundles, due to the fact that some of the large metaxylem cells in the conjunctive parenchyma extend so deep towards the central pith, that they look as if they are protuberances of the conjunctive parenchyma; but here they are not abstricted from it in the central pith as medullary bundles. The pith cells are thin-walled parenchyma. A few raphide and mucilage cells occur in them.

Caryota urens L.

This Fish-tail palm is found in sub-Himalayan tracts, Assam, Upper Burma and in evergreen forests of Konkan, Kanara and Ceylon.

A T.S. of an underground portion of root, about 4 m.ms. in diameter, has large squarish lignified cells in the limiting layer and a broad band of lignified hypodermis filled with dark-brown contents (Fig. 34, 35). The cortex consists of thin-walled round cells with small triangular spaces and 3-4 rows of spindle-shaped or oval air cavities in the middle. Fibre bundles of *Phoenix* type with stegmata are present throughout the cortex. Several raphide sacs and a few mucilage and tannin cells are also present. Endodermis is one-layered conforming to the "C"-type of Russow. Pericycle is one-layered made up of large cells. About 37 strands of xylem and phloem are radially arranged in a highly sclerotic ring of conjunctive parenchyma extending from pericycle to centre in several layers. Xylem consists of a large metaxylem cell around which lie 1-2 secondary xylem and protoxylem cells. Phloem strands are more or less circular. Some of the large metaxylem cells lie deep in the interior in conjunctive parenchyma abutting on the central pith, nearly $480 \times 320\mu$. It consists of round thin-walled cells with raphides sacs, and air cavities here and there. Only one medullary bundle of hydrocentric type is seen in it.

Caryota mitis Lour.

A T.S. of an adult root, about 7,040 m.ms. in diameter, of this elegant species found in Burma, Malaya, Penang, Andaman Islands, is similar to that of *C. urens* (Figs. 36, 37). Epiblema, hypodermis, fibre bundles and cortex are similar to those in that species except that the air cavities lie in 2-3 rows and are larger. There is a complete ring of stone cells in the inner cortex. Raphide sacs, mucilage and tannin cells are as in *C. urens*. The central vascular cylinder is also similar to that in it, but the pericycle is two-layered (Fig. 37). The stele consists of 38 arches of xylem and phloem strands. Many metaxylem cells lie in the conjunctive parenchyma extended towards the central pith. The central pith nearly 1120μ has air cavities and one medullary bundle of hydrocentric type.

Chrysalidocarpus lutescens H. Wendl.

This yellow *Areca* palm, common in Madagascar and Bourbon Island is often cultivated in India.

A T.S. of an aerial portion of an adventitious root, about 7.760 m.ms. in diameter, has epiblema with dark contents. Lignified hypodermis has also dark contents (Fig. 38). The cortex has thin-walled round cells and 2-3 rows of small air cavities. A large number of fibre bundles of *Areca* type are present in the cortex. A few sclereids, raphide sacs, mucilage and tannin cells are also present in it. An incomplete ring of pigment cells and large fibre bundles are present in the outer cortex. Endodermis is of the "C"-type; pericycle is double. The stele is circular in young underground parts but is thrown into folds later as in *Areca* sp. described by Drabble (1904). There are about 84 xylem and phloem strands, some of which are "V"-shaped and others "I"-shaped. If the xylem arch is "V"-shaped, phloem lies in the fork of "V" of xylem. At the apex of the arch there is generally a large cell or two of metaxylem surrounded by thin-walled parenchyma. The stele lies in a much inwardly convoluted conjunctive parenchyma. Some of the convolutions on the inner side of the sclerotic parenchyma include metaxylem cells, which protrude in the central pith and are cut off later as medullary bundles. This is especially noticeable where the ring of pith becomes convoluted and looks as if it is going to break. The splitting up of stelar ring below a depression is achieved in two ways: by pushing a phloem strand down below the notch or by the fusion of two protoxylem groups of two adjacent xylem strands into an inverted "V" of xylem as described by Drabble (1904). In other cases the same result was achieved by pushing the xylem strand into conjunctive tissues, two adjacent phloem strands fusing into an inverted "V" of phloem, below which lies a xylem strand or sometimes a phloem patch. This method of breaking up of the stele by pushing xylem strand into pith and consequent fusion of the phloem strands to form a polystelic condition has not been described by Drabble (1904) in his material. Should this process of breaking and separation of vascular bundles continue, it will ultimately result in a polystelic condition described by Cormack (1896) and Drabble (1904) in species of *Areca*. But no such polystelic condition was noticed here. The central pith in a root is 2560μ , and thin-walled. Fibre bundles of *Areca* type and air cavities are also present in the pith. 3-4 medullary bundles of hydrocentric type are present in the pith. A condition somewhat similar to one described here is known to exist in the roots of *Areca*, *catechu*, *Archontophoenix cunninghamii*, *Howea belmoreana*, *Dyopsis madagascariensis*, etc.

Hyophorbe amaricaulis Mart.

In this palm of Mauritius the underground roots are large and thick. A T.S. is about 7.920 m.ms. in diameter, and has epiblema, hypodermis and cortical cells similar to those in *C. lutescens*, except that the air cavities in it are arranged in 2-3 rows (Fig. 39). Numerous sclereids are present in the cortex (Fig. 41). Raphide sacs, mucilage and tannin cells are also present. A complete ring of sclereids and pigment cells are present in the inner cortex. The stele consists of 43 arches of xylem and phloem. The central vascular cylinder has single-layered endodermis and two-layered pericycle. The xylem strands are "I"- or "V"-shaped. The phloem strands are situated in the arms of "V"-shaped xylem while the larger phloem strands lie by the side of xylem. The conjunctive tissue is highly lobed towards the central pith, 1350μ . Sclereids and fibre bundles of *Areca* type, and one medullary bundle with a metaxylem cavity are present in the pith (Fig. 40).

A T.S. in the distal part of a root, 14.080 m.ms. in diameter has epiblema, hypodermis and cortex similar to that in the last species (Fig. 42). Air cavities are arranged in 6-7 rows. Sclereids (Fig. 41), raphide sacs, mucilage and tannin cells are also present in the cortex. A complete ring of sclereids is present in the inner cortex. The stele consists of 87 arches of xylem and phloem. The central vascular cylinder is irregularly circular. The central pith, 5440μ , consists of thin-walled round cells. Here and there elongated cells are present in the central pith.

There are as many as 16 medullary bundles with metaxylem cavities. At places they are fused by their conjunctive parenchyma. Drabble (1904) while describing this species does not mention the presence of elongated cells in the pith or the fibre bundles of *Areca*-type.

Oreodoxa regia Kunth.

In this widely cultivated Jamaican plam, a T.S. of an adult root, 6.080 m.ms. in diameter, is circular and has a single layer of square lignified cells of limiting layer (Fig. 43). Hypodermis is very extensive, differentiated into three zones. The outer lignified zone with polygonal cells is filled with dark contents; below this lies the middle zone of unlignified cells without any contents but with raphides, and the inner zone of lignified cells also having raphide sacs. The cortex consists of thin-walled round parenchyma with intercellular spaces. Air cavities in the middle cortex are large and radially elongated. The inner cortex consists of a continuous wavy ring of stone cells and 3 layers of oval elongated parenchyma abutting on the endodermis. An incomplete ring of pigment cells in the inner cortex, raphide sacs, mucilage and tannin cells are also present in the cortex. The endodermis is of "U" type and pericycle is one-layered. About 39 arches of xylem and phloem are present and lie radially in the sclerosed conjunctive parenchyma extending nearly to the centre of the root. Xylem strands are arranged in "V"- or "I"-shaped manner including phloem and have large lignified cells at their apex lying centripetally. The central pith, 800μ , is star-shaped and has a few raphide sacs and mucilage cells. Drabble (1904) has described medullary strands in this species but they were not seen in the present material.

Sometimes some of the adventitious roots arising from pericycle at a higher level in the root-bearing region of the tree trunk penetrate downwards in the cortex, probe into tissues of another root, also going down in the cortex, and the two together emerge from the stem and enter soil as a conjoint root.

Cases of two or more roots traversing the cortex of an older root were also seen. Such confluence of roots going down the cortex is quite common in plams and in monocots such as *Asphodelus tenuifolius* and in some *Leguminosae*. Similar root structure having roots within roots was seen in *Phoenix sylvestris*, *Howea* (*Kentia*) *Forsteriana*, etc., and to the best of our knowledge has not been described so far.

Howea Belmoreana Becc.

This palm from Lord Howe's Island is cultivated in India. A T.S. of an underground adventitious root, about 6.720×5.840 m.ms. has lignified limiting layer and hypodermis (Fig. 44). The cortex consists of thin-walled oval cells, but at places they are elongated measuring $160\mu \times 128\mu$. The middle cortex is extensive and is traversed by irregularly shaped 1-2 rows of air cavities lysignously formed. Fibre bundles of *Areca* type become progressively larger and larger in size centripetally and are scattered throughout the cortex. The stegmata are present around fibre bundles, and numerous sclereids occur in the cortex. Sclereids and pigment cells form an incomplete ring in the inner cortex. The stele is complete in young roots with a diameter of 2 m.ms., but at its both ends the cortical parenchyma is joined to the pith in the centre. In still larger roots, the stele is broken at many places and has many gaps. Endodermis is one-layered, its cells belong to "C" type of Russow and have no passage cells. The continuity of endodermis is broken at places due to breaking up of the stelar region. The inner cortex consists of sclereids with 23 layers of intervening parenchyma between endodermis and sclereids. The stele consists of 30 xylem strands and 31 phloem strands. Phloem strands in the stele, where presumably split in a particular phloem strand occurs, is divided into two strands, one on either side of the gap. The xylem and phloem strands lie radially in sclerotic conjunctive parenchyma and are much

lobed internally. The xylem groups are single and "I"- or "V"-shaped. Sometimes phloem strands occur in two series lying in the conjunctive parenchyma. Secondary xylem cells form the base of "V"-shaped xylem strands and lie far away from the sub-pericyclic xylem strands. They are surrounded by a layer of lignified parenchyma, the cells of which are rather small. The central part of root is occupied by pith about $2640 \times 2680 \mu$. Fibre bundles of *Areca*-type, sclereids, raphide sacs and occasionally a medullary bundle, are seen in it. A few air cavities and mucilage cells are also present in the central pith. The roots of this species resemble strongly with those of *Dyopsis madagascariensis* and *Cyrtostachys rendah* described by Drabble (1904).

Cyrtostachys rendah Rl.

This Sumatran gregarious palm has underground root, about 5.360 m.m. in diameter. Its main features are similar to those of *H. Belmoreana*. Epiblema, hypodermis and cortex are similar in them but the cortical cells are thin-walled and round (Fig. 45). Air cavities raphide sacs, tannin and mucilage cells are also similar. A large number of fibre bundles of *Areca*-type and sclereids are present in the cortex. The stele consists of 35 arches of xylem and phloem. The central vascular cylinder is circular. Endodermis is one-layered and its cells belong to "C" type of Russow. Pericycle is also one-layered. The xylem strands are "I"- or "V"-shaped with large metaxylem cells pointing towards pith. Phloem strands of two types are arranged in two series. Smaller ones, $52 \times 36 \mu$, and larger ones $220 \times 20 \mu$, alternate regularly with xylem strands. This type of phloem arrangement was not observed by Drabble (1904) in this species. The conjunctive parenchyma in older roots is completely lignified and lobed towards the centre. Two types of medullary bundles are present in the pith. Two of the medullary bundles have only metaxylem cavity and are hydrocentric, while the other two are leptocentric. The central pith, 1680μ , has air cavities, fibre bundles of *Areca* type, sclereids and a few mucilage cells. Drabble (1904) in his description has mentioned that in large roots, the conjunctive parenchyma is incomplete on one side and its incurved edges are followed by endodermis more deep than usual. The endodermis is, however, finally lost in the common ground tissue. He also observed projecting bays of fibrous tissue together with a phloem strand.

Archontophoenix Cunninghamii W. & Dr.

This Australian palm common in Queensland, Sunday Island, Rockhampton, N. S. Wales, Illowara and Woolongong has adventitious roots about 5.230 m.ms. in diameter. It is circular in outline and has a single limiting layer of square, lignified cells (Fig. 46). Hypodermis is very extensive and is differentiated into three zones. The outer lignified zone has polygonal cells filled with dark contents. Middle zone with 5-6 layers of cells has less contents, and the third inner zone has lignified cells without any cell contents. The cortex consists of round thin-walled cells. The cells are compactly arranged in the outer cortex and have a few raphide sacs, mucilage and tannin cells, and fibre bundles of *Areca*-type. The fibre bundles are surrounded by stegmata. A few sclereids are also present in it. The middle cortex is made up of thin-walled parenchyma in which large radially elongated air cavities lie in 2-4 rows. Mucilage cells, a few sclereids and fibre bundles are also present in it. The inner cortex has a structure similar to that of outer cortex but the parenchymatous cells have small intercellular spaces. The stele consists of 36 arches of xylem and phloem. Endodermis is of "C" type, and pericycle is one-layered. Xylem strands are "I"- or "V"-shaped. The conjunctive parenchyma is sclerosed and highly convoluted towards the central pith, 1200μ wide, and thin-walled. A few raphide sacs, mucilage cells, sclereids and fibre bundles of *Areca*-type occur in it but there are no medullary bundles.

The distal part of the root measures 1.100 m.m. in diameter and has a similar structure; but the stele is irregular and has notches. It also shows signs of breaking open as in *Howea Belmoreana*. At places xylem is exarch in the vascular cylinder and at other places endarch (Fig. 47).

There is one hydrocentric type of medullary bundle in the central pith. Xylem strand in the medullary bundles has large metaxylem cells and a few of protoxylem (Fig. 51).

The distal part of the same root, about 12.320×10.720 m.ms. in diameter, has similar epibema and hypodermis, but the epibema is broken up at places by thin-walled spongy tissue with distinct intercellular spaces forming pneumathodes. The cortex consists of thin-walled round cells, but at places strips of elongated cells run irregularly in the cortex. The elongated cells measure $240 \times 60\mu$. Air cavities are large and are arranged in 2-3 rows. *Areca*-type of fibre bundles, sclereids, mucilage cells and tannin cells are also present. Endodermis and pericycle are as before. The stele breaks to form a polystele. The xylem and phloem strands in each stele are radially disposed in lignified conjunctive tissue. The xylem strands are "I"- or "V"-shaped, exarch or endarch here and there. Some phloem strands are small and some are large, arranged at places in two series. They are "U"- or "V"-shaped and are normally oriented or inverted. Wherever a phloem strand becomes "U"- or "V"-shaped, it indicates a point at which the stele breaks as in *H. Belmoreana*. Due to polystely, ground tissues of the central pith and cortex become continuous and numerous fibre bundles of *Areca*-type, sclereids, strips of elongated tissue, air cavities, mucilage cells and raphide sacs occur in them. In the central pith, 4320μ , different types of medullary bundles are present. One or two medullary bundles have a metaxylem cell only (Fig. 47). Others have metaxylem cells and a few protoxylem cells (Fig. 51). In the third type protoxylem lies on both the sides of a metaxylem cell. (Fig. 47). In the fourth type there are distinct xylem and phloem strands. Metaxylem in such xylem strands lies in the centre, and protoxylem lies either centripetally or centrifugally. Three phloem strands lie under a common sheath and the whole structure looks like a small stele (Fig. 49).

Areca catechu L.

The Betel Nut Palm has large pull roots. A T.S. in the proximal part of an adventitious root is 9.320 m.m. in diameter and has one-layered piliferous layer with lignified, square cells. Below it lies lignified hypodermis differentiated into two parts,—the outer filled with dark brown contents, and the inner without them (Fig. 52). The middle cortex is very extensive having an outer region in which numerous small irregular, round fibre-bundles of *Areca*-type are closely packed, and an inner region in which very large fibre bundles lie scattered apart (Fig. 55). 2-3 series of large lysigenous air-cavities occur in it and abut on the inner cortex. A broken ring of sclereids and an incomplete ring of pigment cells occur in the inner cortex. Besides this a number of sclereids are scattered between fibre bundles throughout the cortex. Raphide sacs are numerous and a few mucilage cells are present in the cortex. The endodermal cells belong to "C" type and are followed by one layer of large pericycle cells. According to Drabble (1904) pericycle is two-layered in small roots. The stele consists of 80-90 arches of xylem and phloem and lies in a continuous mass of conjunctive, thick-walled, lignified parenchyma. It is thrown into rays or bays enclosing islands of xylem and phloem below pericycle. Xylem groups are either "I"- or "V"-shaped. Each xylem strand consists of 4-5 cells arranged in radial plates and terminates in a relatively very large metaxylem cell which constitutes the apex of "V"-shaped xylem strand. Such large metaxylem cells are completely included in the sclerosed conjunctive parenchyma. They generally have a single layer of thin-walled, unligified paren-

chyma around them. The conjunctive tissue is thrown into numerous zig-zag lobes on its inner margin. The central pith, 3520μ broad, contains numerous fibre bundles, sclereids, raphide sacs, a few lysigenous air cavities and nearly seven medullary bundles of different size, all hydrocentric.

A T.S. in the distal part of the same root, 13.280 m.m. in diameter, has similar epiblema, hypodermis, cortex, endodermis, pericycle, xylem, phloem, and conjunctive tissue as in the proximal part of the same root, but the stele is broken up to form a polystele (Figs. 53, 54). The adult root thus resembles with the adult root of *Archontophoenix Cunninghamii*. In a stele prior to the breaking up into a polystele, phloem strand becomes "U"-shaped. The broken convoluted bit of the stelē forms an independent arch. The species agrees in this respect with *H. Belmoreana* and *A. Cunninghamii*. The central pith, 5×8 m.m., is continuous with the parenchyma in the cortex and has a similar structure. A few medullary bundles of different kinds are present in it. The abstricted medullary bundles lie in the gaps formed by the separation of stelar arches. Some of them lie scattered even in the central pith.

In the medullary bundle shown in Fig. 56, there is a large metaxylem cell and a single phloem strand encircled by an oval ring of lignified conjunctive parenchyma.

A second medullary bundle is shown in Fig. 57. Here two xylem strands have been united by a large metaxylem cell. The two phloem strands lie on flanks opposite to each other. They are completely encircled by sclerenchymatous conjunctive parenchyma.

A third medullary bundle is shown in Fig. 58. Here a large metaxylem cell in the middle and a distinct strand of secondary xylem lie centripetally. There are three phloem strands, two on two sides of the isolated xylem strand and one above the large metaxylem cell. Both the xylem and phloem strands are enclosed in strong lignified conjunctive parenchyma.

These three types of medullary bundles always lie in the vicinity of stelar gap, from which they are abstricted in the process of splitting up of the vascular strands into loops. They are different from the ordinary medullary bundles in the central pith and have 8-9 layers of lignified sclerenchyma around a single metaxylem cell without any phloem. All such irregularities ultimately result in the so-called polystelic condition, previously described for roots in *Areca* by Cormack (1896) and Drabble (1904). Further disintegration of these islands of stelar pockets to include small medullary bundles as in *Iriartes* was also seen in the present study. Presumably, these occur in roots arising in the cortex at a much higher level of a tree trunk.

Elaeis guineensis Jacq.

This oil palm of Africa has a jacket of roots at the base of the tree trunk as in *Phoenix* or *Borassus*. There are two kinds of roots in this palm, underground and subaerial. According to Yampalosky (1924) underground roots are attached to the base of tree trunk and radiate in all directions and grow over a considerable distance. The subaerial roots arise above the ground from older tree trunks, generally a meter above the ground in discontinuous regions. Most of the adventitious, subaerial roots ultimately penetrate into the soil.

A transverse section of an underground portion of a root is about 4.960 m.m. in diameter. The limiting layer has square lignified cells, broken at places due to pneumathodes. Hypodermis consists of lignified cells filled with brown cell contents (Fig. 59). The cortex consists of thin-walled round cells having numerous raphide sacs in the outer cortex and air-cavities in the middle cortex. Several oil sacs occur throughout the cortex. Inner cortex consists of a complete ring of stone cells lying over a few layers of oval, transversely elongated parenchyma abutting on endodermis. Endodermis and pericycle are one-layered. The stele

consists of 41 arches of xylem and phloem lying radially in conjunctive parenchyma highly lignified towards the central pith. Xylem strands are "I"- or "V"-shaped. The phloem strands are of two kinds : the smaller ones measure 48μ and lie in the fork of "V"-shaped xylem and the large ones, $160 \times 48\mu$, lying by the side of "I"-shaped xylem. The central pith 640μ , forms a zig-zag patch having raphide sacs and oil-containing cells. No medullary bundles occur in the central pith.

Attalea speciosa Mart.

This Brazilian palm is occasionally cultivated in India. Its adventitious roots are about 8,000 m.m. in diameter, and have lignified epiblemma with squarish cells having dark contents (Fig. 60). Hypodermis is lignified and is differentiated into an outer zone with dark contents and an inner zone rather clear and with raphide sacs. The large cortex consists of thin-walled, round cells and air-cavities arranged in 2-3 rows in the middle. There is an incomplete ring of tannin cells in the inner cortex. Raphide sacs, mucilage cells and tannin cells are also present in the cortex. The stele consists of 50 arches of xylem and phloem lying radially in lignified conjunctive parenchyma. The xylem strands are "I"- or "V"-shaped. The central pith, about 1.2 m.m., consists of thin-walled round cells. Air cavities, a few raphide sacs and mucilage cells occur in it.

Cocos nucifera L.

A T.S. of aerial portion of an adventitious root, about 10 m.m. in diameter, has lignified epiblemma, and 6-7 layers of lignified hypodermis filled with dark contents (Figs. 61-63). The cortex consists of thin-walled large parenchymatous cells having small intercellular spaces. Several raphide cavities (Fig. 62) and mucilage cells are present in the cortex. Many air cavities of various shapes occur in 2-3 rows in the middle cortex. An incomplete ring of pigment cells is present in the inner cortex. The endodermal cells conform to "C" type of Russov with passage cells. Pericycle is thin-walled. Cells of the conjunctive parenchyma below pericycle are not thick-walled. There are about 50 arches of xylem and phloem radially disposed, mostly in the unlignified conjunctive tissue. Xylem strands are "I"- or "V"-shaped, phloem lying in the arms of "V". The terminal part of xylem strand towards interior is occupied by a large metaxylem cell encircled by a layer or two of thin-walled cells in the rest of the lignified conjunctive parenchyma. A broad patch of pith, 1760μ , has convoluted margin towards the centre. Central parenchyma contains raphide sacs and a few mucilage cells. It also includes two medullary bundles of hydro-centric type (Fig. 63). The two medullary bundles are noticeable in thick roots also. Drabble (1904) observed one only.

Cocos plumosa Hook. f.

A T.S. of adult adventitious root about 9,600 m.m. in diameter has a structure similar to that in *C. nucifera*, except that, air cavities here are arranged in 2-3 rows. The cortical cells get lignified and there is a complete ring of stone cells in the inner cortex (Fig. 64). The stele consists of 75 arches of xylem, and phloem embedded in the conjunctive parenchyma which is thin-walled towards pericycle and lignified towards central pith. The xylem strands are "I"-, "V"- or "Y"-shaped and phloem strands oval, rarely "U"-shaped. Tyloses are present in metaxylem cells. The central pith, 2 m.m. broad, has a few raphide sacs, mucilage cells and air cavities. One or two medullary bundles lie in it, each having metaxylem phloem strands.

Martinezia caryotaefolia Humb. & Kth.

This palm native of Brazil, Peru and Bolivia is occasionally grown in Indian gardens.

A T.S. of the proximal part of adult adventitious root is about 5 m.m. in diameter. It has lignified epiblema and lignified hypodermis filled with dark contents. The cortex consists of thin-walled, oval or round cells, with inter-cellular spaces. The air cavities in the middle cortex are arranged in 2-3 rows. Raphide sacs, mucilage cells and tannin cells are also distributed in the middle cortex. A large number of fibre bundles of *Phoenix*-type are present throughout the cortex. A complete ring of stone cells is present in the inner cortex. Vascular cylinder is circular. Endodermis conforms to "C" type of Russow. Pericycle is one-layered. The stele consists of 35 arches of xylem and phloem and lies embedded in lignified conjunctive parenchyma. The central pith, 450μ , consists of thin-walled round cells without any medullary bundles.

A transverse section of the distal part of the same root, about 84.80 m.m. in diameter, has similar epiblema, hypodermis and cortex as described above (Fig. 65). There is no ring of sclereids in the inner cortex. The stele is broken up to form polystelar groups. Each stelar group has endodermis and pericycle. Xylem strands are "I", "V", or "Y"-shaped. The phloem strands are of three kinds: small nearly 48μ ; they lie in the forks of "V"-shaped xylem: two large strands, $192 \times 32\mu$ which lie by the side with the "I"-shaped xylem; and the crescent-shaped ones formed by the fusion of phloem strands which engulf a few protoxylem groups. At places phloem strands are arranged in two series as in *Howea Belmoreana*, *Archotophoenix Cunninghamii* and *Areca catechu*. Due to polystelic nature, the ground tissue of the central pith, 1920μ , becomes continuous with that of the cortex and has similar type of raphide sacs, mucilage cells and tannin cells. Fibre bundles in the pith are of *Areca*-type. 6-7 medullary bundles with metaxylem cells are also present in the central pith. At places they get fused with one another and also with fibre bundles of *Areca* type.

The breaking up of the stele into polystely is exactly similar to that in *Howea Belmoreana*, *A. catechu* and *Archotophoenix Cunninghamii*. Drabble (1904) had not noticed polystele, medullary and fibre bundles in his material of this species.

Bactris major Jacq.

A T.S. of an adult adventitious root about 3.440 m.m. in diameter has one-celled lignified epiblema and a large band of lignified hypodermis with yellow, dark contents (Figs. 66, 67). The cortex consists of thin-walled oval or round cells. Air cavities in the middle cortex are large, lysigenously formed, and arranged in 2-3 rows. Cells in the middle cortex are slightly large, nearly 72μ . Raphide sacs, mucilage cells, sclereids and tannin cells are present in the cortex. A complete ring of pigment cells is present in the inner cortex.

The stele is circular and consists of 28 arches of xylem and phloem embedded in conjunctive parenchyma. The conjunctive parenchyma is lignified towards the central pith but thin-walled towards the pericycle (Fig. 67). The xylem strands are "I", "V", or "Y"-shaped. The phloem strands are of two kinds: small ones, $40 \times 40\mu$, lying in the fork of "V", or "Y"-shaped xylem, and large ones, $200 \times 40\mu$, lying by the side of "I"-shaped xylem (Fig. 67). The central pith $200-320\mu$, consists of thin-walled round cells with a few tannin and mucilage cells.

Nipa fruticans Wurumb.

A transverse section of the sub-merged part of an adventitious root, about 3.920 m.m. in diameter, is circular and has a single layer of epiblema, made up of

thick-walled, unligified cells with their tangential walls cuticularised (Figs. 68, 69, 70). There is a band of 5-6 layers of lignified hypodermis. The cortex is very extensive and spongy, made up of thin-walled oval or round cells with small triangular spaces. The whole of the middle cortex is traversed by radially elongated schizogenous air-cavities formed by the chains of cells (Figs. 68, 70). The raphide sacs occur in the inner and outer parts of the middle cortex. Mucilage cells are restricted to the middle cortex. The inner cortex consists of small round parenchymatous cells compactly arranged. The endodermis is entire, its cells being lignified on radial walls only (Fig. 69). Pericycle is one-layered. Stele is composed of 25 arches of xylem and phloem radially distributed in the conjunctive tissue (Fig. 69). The conjunctive parenchyma below pericycle is thin-walled, but in its lower part towards centre, it is thick-walled. Large secondary xylem cells lie partially embedded in the lignified conjunctive parenchyma, and partially above it in the thin-walled conjunctive parenchyma. The xylem groups are "I"-shaped and the small phloem strands are spherical. The central part of root is occupied by pith, 240μ . The intercellular spaces are triangular. A few mucilage cells and raphide sacs occur in the central pith.

The stele is small, and cortex is traversed by numerous schizogenously formed air-cavities lined by chains of radially elongated cells, fibres or other mechanical strands are developed in it. These features constitute distinctive characters of the roots of this tidal palm. They show a strong resemblance with the structure of roots of a fossil palm from the Deccan Intertrappean series described by Sahni (1938) under the name of *Rhizopalmoxyylon indicum*.

V. IMPORTANT ANATOMICAL FEATURES OF PALM ROOTS

The present study deals with the structure of adult roots in palms arising from stem in the root-bearing region of a tree-trunk in 35 species belonging to all important sub-families of Palmae except Mauritiaceae of which no material was available. The stelar structure of a palm root as it traverses through cortex of a stem, from which it arises, is complex initially but gets simplified later towards the growing point of the root, as it comes out of the tree-trunk. It is a case of anatomical inversion as opposed to that of condensation in peduncles where the complexity of anatomical structures increases from base to apex and vascular bundles and other tissues tend to fuse together more and more. The principal features of palm roots are as follows:—

(1) *Epiblema*.—In most palm roots, epiblema consists of cuticularised cells, which become lignified later and constitute a limiting layer on the outer cortex. Hypodermis is lignified and is often made up of three zones: the outer zone filled with a dark substance, the tannin, the middle zone with thin-walled cells, and the inner zone having thick lignified cell-walls but no cell contents. *Nipa fruticans* is exception to this, as, in it the outer zone of hypodermis is parenchymatous and is without any cell contents or tannins. In all palm roots a few raphide sacs do occur in the inner zone of epiblema. In *Hyphorbe amaricaulis* and *Oreodoxa regia* a large layer of thin-walled cells lies between the two layers of hypodermis and is not lignified. It contains no pigment cells.

(2) *Cortex*.—Below epiblema lies cortex having three parts, outer, inner and middle, easily recognisable. Outer cortex forms a continuous band of 3-6 layers of cells in all the palm roots. Raphide sacs and pigment cells occur in it. It is extended up to the inner cortex lying on endodermis. In between the outer and inner cortex are several rows of parenchymatous cells containing air cavities. They constitute the middle cortex. Middle and inner cortices show a good deal of variation in different palms. These air-cavities are formed lysigenous in most palms, except in *Nipa fruticans* where they are formed schizogenously. They occur in the lower part of the middle cortex in *Rhapis flabelliformis* and *Chrysalidocarpus lutescens*.

(Figs. 11, 38). They are very small and occur throughout the middle cortex in *Livistona chinensis*, *Hyphaene indica*, *Borassus flabellifer*, *Caryota urens*, *Hyophorbe amaricaulis*, *Howea Belmoreana*, *Archontophoenix Cunninghamii*, *Areca catechu* and *Cocos nucifera*. They are very large and radially elongated in *Phoenix paludosa*, *Bactris major* and *Nipa fruticans*. In the rest of palms studied they were irregularly distributed. The extent of air-cavities in roots varies from palm to palm and even in the same palm it depends upon the kind and position of root in the soil as shown by d'Almeida and Correa (1949).

The inner cortex consists of 2-5 layers of oval, transversely elongated, cubical parenchymatous cells dividing radially. These layers lie directly on endodermis. Generally a few pigmented cells occur in the inner cortex (Fig. 20). The pigmented cells abut on endodermis and form a continuous band in *Corypha umbraculifera*, *Caryota mitis*, *Hyophoebe amaricaulis* and *Bactris major*; sclereids are generally associated with these pigmented cells on the endodermis and occur in groups as isolated nests of 3-7 cells (Fig. 9-sc). In *Trachycarpus martiana*, *Caryota mitis*, *Hyophorbe amaricaulis*, *Oreodoxa regia*, *Elaeis guineensis* and *Cocos plumosa* they form a complete circle of sclereids (Figs. 10, 36, 41, 59, 64).

By far the most important feature of the cortex of palm roots is fibre bundles. They are of three kinds: (1) *Phoenix*-type having a circular outline as in *Phoenix sylvestris*, *Phoenix rupicola*, *Phoenix paludosa*, *Phoenix zeylanica*, *Phoenix dactylifera*, *Corypha umbraculifera*, *Hyphaene indica*, *Caryota urens*, *Caryota mitis*, and *Martinezia caryotaefolia*. (2) *Areca*-type.—The fibre bundles in this type have polygonal shape as in *Chrysalidocarpus lutescens*, *Howea Belmoreana*, *Cyrtostachys rendah*, *Archontophoenix Cunninghamii* and *Areca catechu* and (3) *Hyophorbe*-type in which there is no grouping of cells, but they occur as individual fibre cells: sometimes they lie scattered throughout the cortex as in *Hyophorbe amaricaulis*, *Livistona chinensis* and *Latania verscaffeltii*. In a few exceptional cases, fibre bundles of *Areca*-type are also present in the central pith only e.g., in *Corypha umbraculifera*, *Sabal Adansonii*, *Hyophorbe amaricaulis* and *Martinezia caryotaefolia*. In *Sabal serrulata* the fibres are of the *Hyophorbe* type but they lie in the central pith only. In *Corypha umbraculifera* and *Martinezia caryotaefolia* the fibre bundles in the cortex are of *Phoenix*-type while those in the central pith are of *Areca*-type. In *Hyophorbe amaricaulis* there are loose sclereids in the central pith and also some fibres of the *Areca*-type.

(3) *Endodermis*.—In most of the palm roots cells of the endodermis are lignified on their lateral and inner walls (Fig. 20) and they conform to the "C"-type of endodermal cells of Russow except in *Calamus rotang* and *Calamus tenuis* where, due to lignification all round the cell walls, they form the "O"-type of Russow. Distinct Casperian bands are found in *Hyphaene indica*, *Borassus flabellifer* and *Nipa fruticans* (Fig. 69). Passage cells are observable in roots of most palms.

(4) *Pericycle*.—This is generally one-layered, rarely two. It is often not lignified, but is very much so in *Rhapis flabelliformis*. Walls of the pericycle cells near protoxylem are generally thin.

(5) *Stele*.—This is very variable in different species and presents some well recognizable types ranging from eustele to the so-called polystele described by Cormack (1896), Drabble (1904) and Bower (1923). The different configurations of stele in palms roots no doubt occur as an adjustment to the expanding and enlarging size of root, which, owing to lack of cambium, is unable to get modified like the root of dicotyledons. The different patterns of stele in palm roots with its conjunctive parenchyma, therefore, form a very characteristic feature of each species as are shown in Figs. 1-70.

It will be seen from them that some additional patterns of stele have been brought out in the present investigation besides those described by Von Mohl (1849), Cormack (1896) and Drabble (1904). They undoubtedly form interesting examples of polysteler types of roots in palms. Cormack (1896) and Drabble (1904)

have also described typical eustele and intermediate types thereof. More complex disintegration of stele occurs in the living palms such as *Iriartes*. A similar case of disintegration has been described by Stenzel (1904) in a fossil palm root comparable with that of the modern species of *Iriartes*.

(6) *Vascular bundles*.—In most of the palm roots, xylem strands are arranged in a single row (Figs. 1, 3, 8, 24, 69), but in *Chamaerops humilis* they lie in more than one row (Fig. 9). They are "I"-shaped or "V"-shaped or both (Figs. 5, 26, 37), except in *Nipa fruticans* where they are all "I"-shaped. In *Areca* they are "Y"-shaped in addition (Fig. 54). The phloem generally forms an oval patch lying by the side of "I"-shaped xylem strands: alternatively it may lie in between two arms of "V"-shaped or "U"-shaped xylem strands (Fig. 5, 26, 37). The phloem strands are present in two series in *Hyphaene indica* and *Borassus flabellifer* (Figs. 28, 31). They are present in two or three series occasionally in *Howea Belmoreana*, *Archontophoenix Cunninghamii*, *Areca catechu* and *Martinezia caryotaefolia*. The phloem strands are "V"-shaped or semicircular and xylem strands lie in them in *Sabal adansonii*, *Howea Belmoreana*, *Archontophoenix Cunninghamii*, *Areca catechu*, *Cocos plumosa* and *Martinezia caryotaefolia* (Figs. 44–65).

Generally there is a large metaxylem cell or cells in a xylem strand. They lie centripetally and extend into the conjunctive tissue which is very much thickened in most of the palms except in *Phoenix sylvestris*, *Corypha umbraculifera*, *Hyphaene indica*, *Borassus flabellifer*, *Hyophorbe amaricaulis*, *Elaeis guineensis* and *Nipa fruticans*. In these palms it is only partially thickened. The extent and the manner of thickening of conjunctive parenchyma differ in different palm roots. In the secondary aerial roots of many palms and in *Sabal serrulata* the conjunctive tissue is completely lignified.

Due to uneven lignification of cells on the inner margin of the conjunctive parenchyma, the inner edge touching central pith looks circular, convoluted or irregularly lobed (Figs. 5, 25, 31). Its outer margin lies below endodermis and is thrown into bays and arms. These bays include xylem and phloem strands (Fig. 9). The convoluted lobes of inner margin include sometimes large secondary metaxylem cells and medullary bundles in the process of getting abstricted in the central pith (Fig. 23). In *Areca catechu* and in *Iriartes* this process results in an extreme condition looking like a polystele or disintegration. Here there are numerous islands of conjunctive parenchyma; and in the midst of each island there is a meristele as described by Cormack (1896) and Drabble (1904). A new feature of palm roots noticed in the present investigation was the breaking up of the vascular ring of stele by splitting up of a phloem strand in *Areca catechu* and *Martinezia caryotaefolia*. Cormack (1896) and Drabble (1904) did observe such breaking up of stele in their material, but there it was by the splitting up of a xylem strand and not of a phloem strand as described here.

(7) *Pith*.—The central part of a palm root consists of unlignified cells except in secondary roots where they get lignified. The extent and shape of central pith are different in different palms due to various degrees of lignification of the conjunctive parenchyma and the presence or absence of medullary bundles in it. It generally contains a few raphide sacs, pigment cells, oil secreting cells, sclereids or their aggregations, aerenchyma and medullary bundles. All these characters are of course not present in all palms. But the medullary bundles form a highly characteristic feature of the roots of *Phoenix sylvestris*, *Phoenix zeylanica*, *Phoenix paludosa*, *Rhapis flabelliformis*, *Corypha umbraculifera*, *Licuala peltata*, *Licuala grandis*, *Livistona chinensis*, *Sabal adansonii*, *Borassus flabellifer*, *Calamus tanuis*, *Caryota urens*, *Caryota mitis*, *Chrysalidocarpus lutescens*, *Hyophorbe amaricaulis*, *Cyrtostachys renda*, *Archontophoenix Cunninghamii*, *Areca catechu*, *Cocos nucifera*, *Cocos plumosa* and *Martinezia caryotaefolia*. In the other palms studied they were absent.

A medullary bundle consists of xylem surrounded by phloem and lignified cells of parenchyma in *Phoenix sylvestris*, *Phoenix zeylanica*, *Corypha umbraculifera*, *Licuala peltata*, *Livistona chinensis*, *Caryota urens*, *Caryota mitis*, *Chrysalidocarpus lutescens*, *Areca catechu* and *Cocos nucifera*. In *Corypha umbraculifera*, *Licuala grandis*, *Hyophorbe amaricaulis*, *Areca catechu* and *Martinezia caryotaefolia* more than one medullary bundles occur in the central pith (Figs. 13, 13, 41, 54, 65). In *Corypha umbraculifera* and *Cyrtostachys renda* the medullary bundles fuse together as they traverse through the cortex vertically downwards. The xylem strand disappears first and then only a small patch of phloem remains, forming leptocentric bundles (Figs. 16, 18).

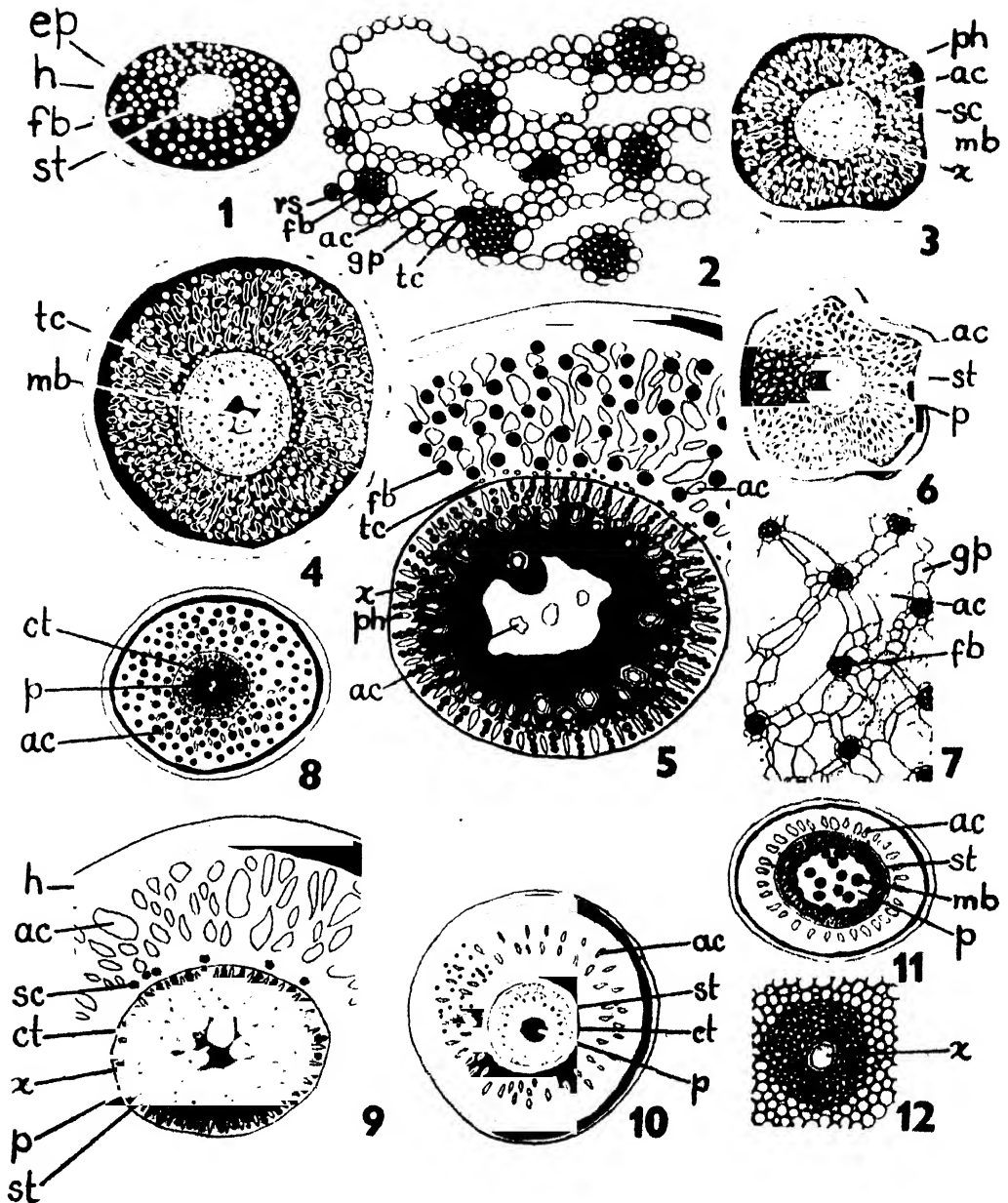
Raphide sacs containing needle-shaped crystals of calcium oxalate are present in all the palm roots in the cortical region, but in *Livistona chinensis*, *Chrysalidocarpus lutescens*, *Hyophorbe amaricaulis*, *Archontophoenix Cunninghamii*, they are present in central pith. Oil glands are present in *Elaeis guineensis* but not in other palms. In the roots of *Hyophorbe amaricaulis* and *Archontophoenix Cunninghamii* the cortical parenchyma consists of elongated cells similar to those found in the stem and peduncles of those palms. This is rather a unique feature of roots of these species. However, there are no elongated cells in the cortex of their root (Figs. 40, 48).

All these characters of the palm roots, especially the medullary bundles, fibre bundles in the cortex, the nests of sclereids abutting on endodermis, pattern of aerenchyma and stele are features of considerable interest and they help in recognising different species of living palms. They can also be so used in the analysis of the artificial genus "*Rhizopalmoxydon*" which is under study by one of us (T.S.M.).

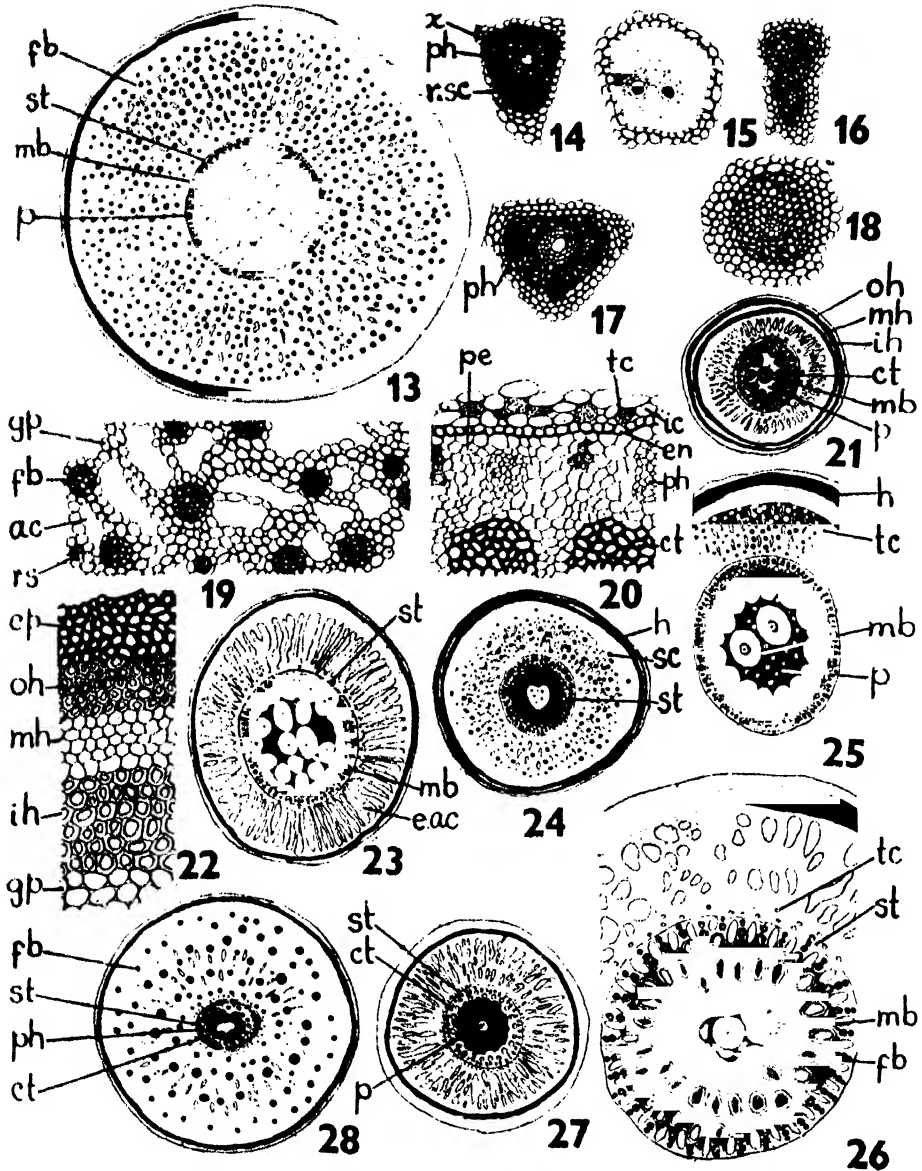
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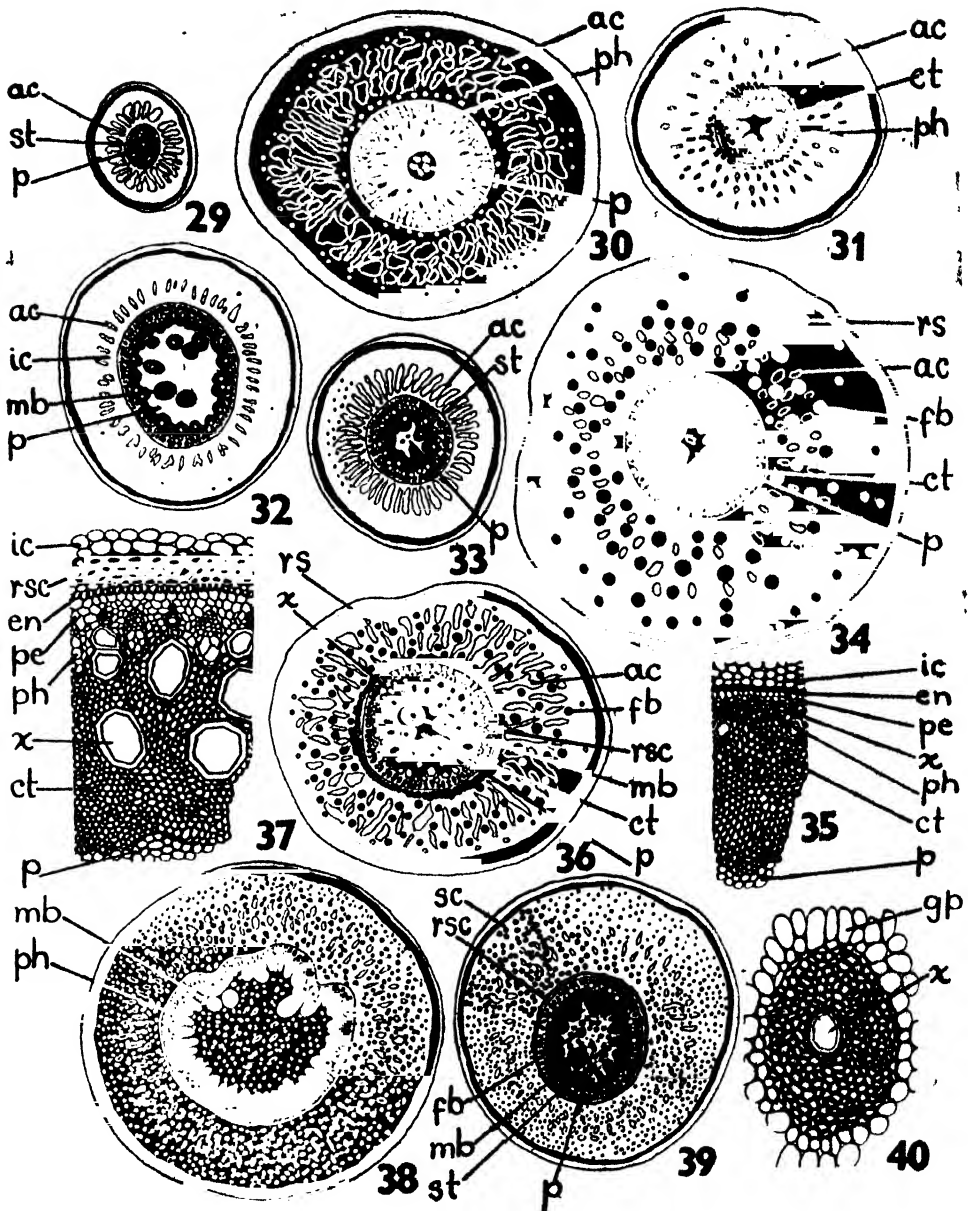
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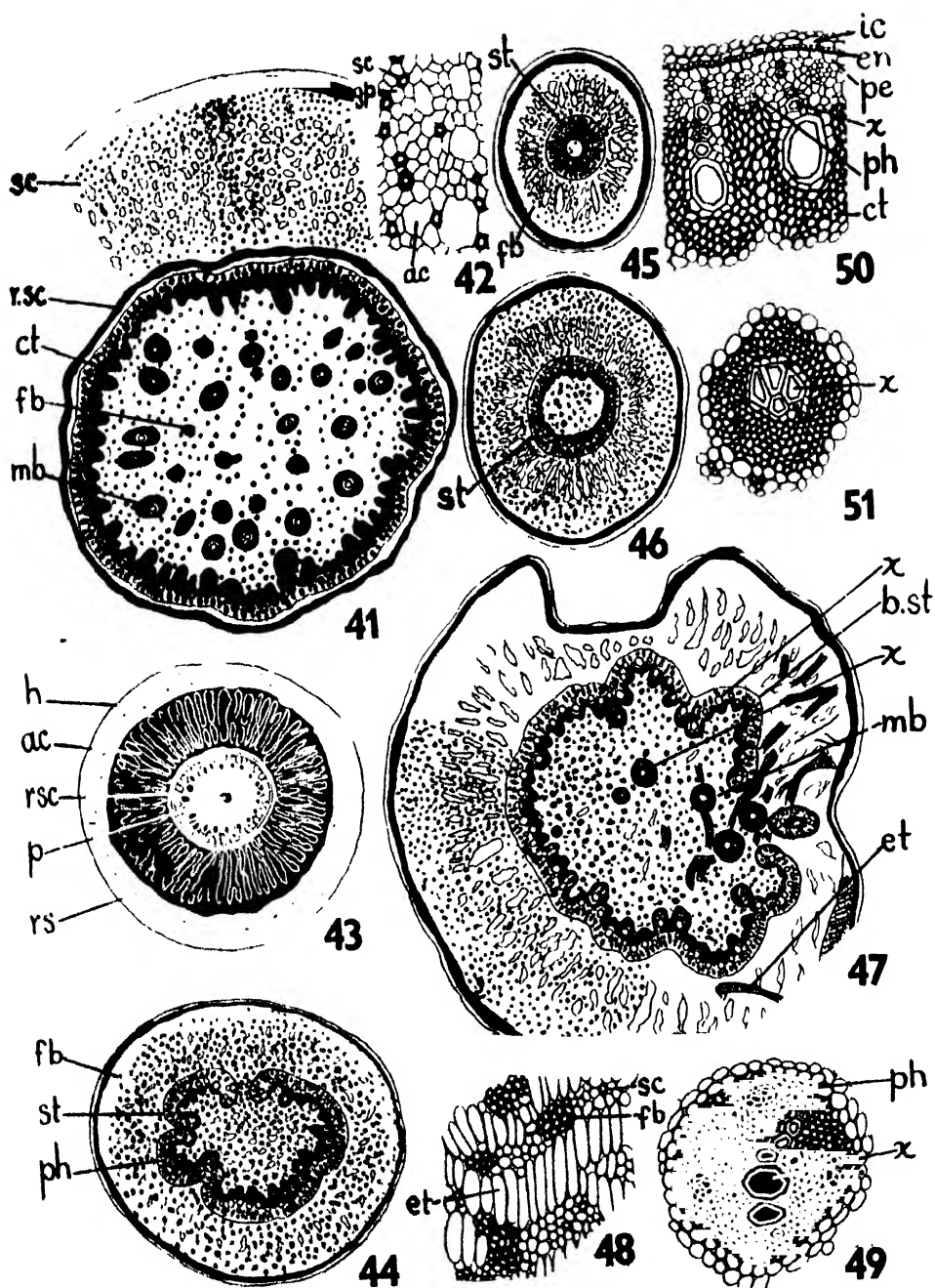
Figs. 1 to 12. T. S. of adult root in *Phoenix*, *Chamaecrops*, *Trachycarpus* and *Rhaps*.
For explanation see text.



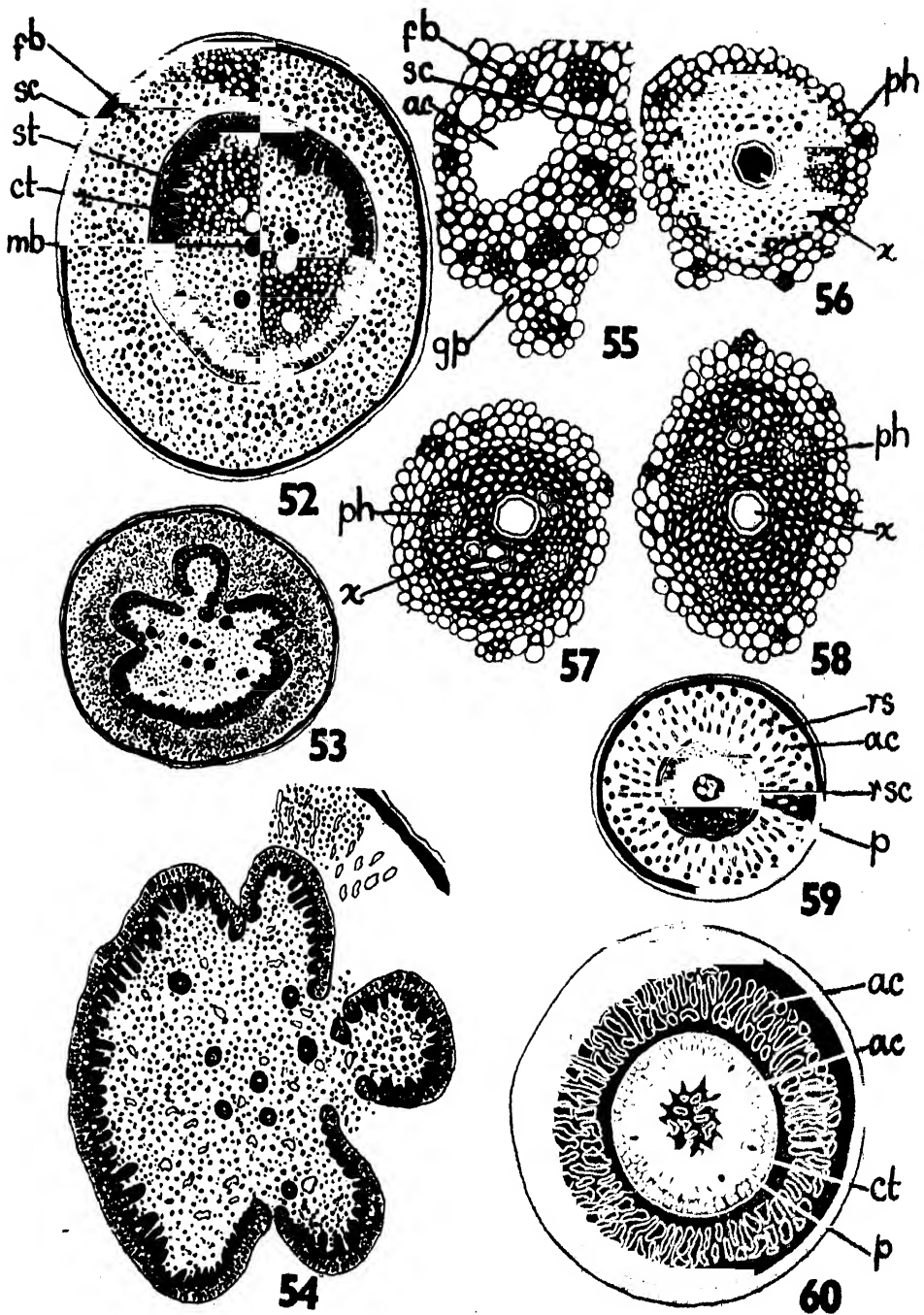
Figs. 13 to 28. T. S. of adult root in *Corypha*, *Licuala*, *Livistona*, *Sabal* and *Hyphaene*.
For explanation see text.



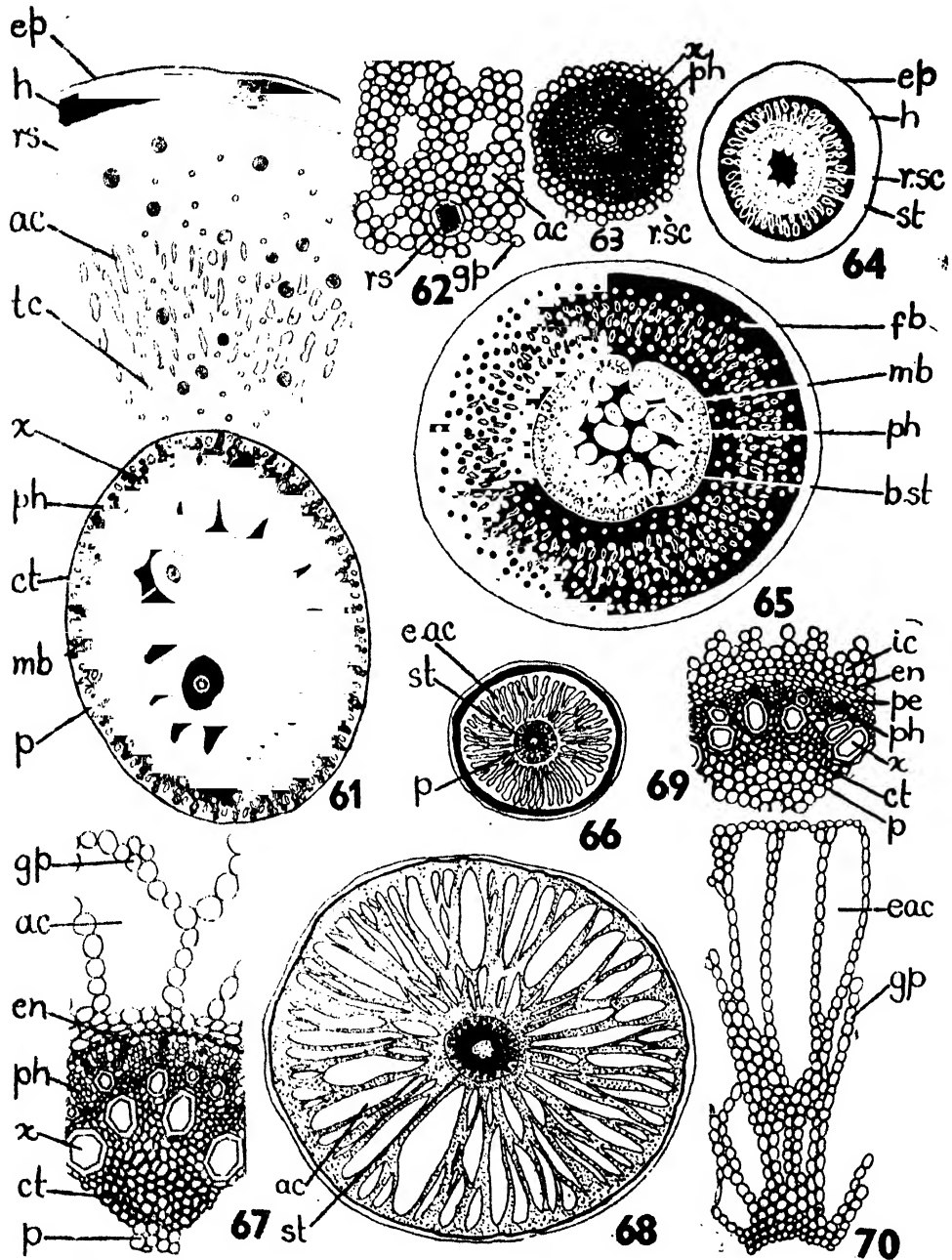
Figs. 29 to 40. T. S. of adult root in *Latania*, *Borassus*, *Caryota*, *Calamus*, *Chrysalidocarpus* and *Hyophorbe*. For explanation see text.



Figs. 41 to 51. T. S. of adult root in *Hyphorba*, *Howea*, *Oreodoxa*, *Cyrtostachys* and *Archontophoenix*. For explanation see text.



Figs. 52 to 60. T. S. of adult root in *Areca*, *Elaeis* and *Attalea*.
For explanation see text.



Figs. 61 to 70. Anatomy of roots in *Cocos*, *Martinezia*, *Baccharis*, and *Nipa*.
For explanation see text.

VII. EXPLANATION OF FIGURES

- Figs. 1-12. Anatomy of roots in *Phoenix*, *Chamaerops*, *Trachycarpus* and *Raphis*. Figs. 1-3. *Phoenix sylvestris* Roxb. Fig. 1. T. S. of aerial root showing: *ep.*, epidermis; *h.*, hypodermis; *f.b.*, fibre bundles, and *st.*, stele with a small pith in the centre $\times 8$. Fig. 2. Cortical portion of an underground root showing: *g.p.*, ground parenchyma; *f.b.*, fibre bundles; *a.c.*, air cavities; *r.s.*, raphide sacs; and *t.c.*, tannin cells $\times 70$. Fig. 3. T. S. of an underground root showing: *x.*, xylem; *ph.*, phloem; *a.c.*, air cavities; *sc.*, sclereids and *m.b.*, medullary bundles $\times 8$. Fig. 4. *Phoenix zeylanica* Trimen: T. S. of adult root showing internal structure: *t.c.*, tannin cells and *m.b.*, medullary bundles $\times 8$. Fig. 5. *Phoenix rupicola* T. Anders. T.S.; of adult root showing: *a.c.*, air cavities; *f.b.*, fibre bundles; *t.c.*, tannin cells; *x.*, xylem *ph*-phloem and *a.c.*, air cavities in the central pith $\times 8$. Fig. 6. *Phoenix paludosa* Roxb. T.S. of adult root showing: *a.c.*, air cavities in the cortex; *st.*, stele and *p.*, pith $\times 8$. Fig. 7. The same: a portion of cortical ground tissue magnified showing *a.c.*, air cavities; *g.p.*, ground parenchyma and *f.b.*, fibre bundles $\times 44$. Fig. 8. *Phoenix dactylifera* L. T.S. of adult root showing internal structure: Note the few *a.c.*, air cavities; pattern of *c.t.*, conjunctive tissue and narrow pith, $p \times 8$. Fig. 9. *Chamaerops humilis* L. Hort. T.S. of adult root showing *h.*, hypodermis *a.c.*, air cavities; *sc.*, sclereids; *st.*, stele in the centre showing xylem, *x.*, in many rows, in *c.t.*, conjunctive tissue, with irregularly lobed strands $\times 15$. Fig. 10. *Trachycarpus martiana* H. Wendl. T.S. of adult root showing *a.c.*, air cavities in two rows and stele with circular conjunctive tissue, *c.t.* towards pith $\times 8$. Figs. 11-12. *Raphis flabelliformis* Ait. Hort. Fig. 11. T.S. of adult root showing air cavities, *a.c.*, stele, *st.*, circular with medullary bundles, *m.b.* in the large central pith $\times 8$. Fig. 12. Medullary bundles with xylem, $x \times 70$.
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SOME ASPECTS OF INTRA-SEASONAL GROWTH-VARIATION IN PLANTS

PART I

by C. V. KRISHNA IYENGAR

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ABSTRACT

A preliminary study of the seasonal and intra-seasonal conditions was taken up in view of their close association with plant's growth. The variation in the daily number of sunspots, and its effect on temperature and other factors have been pointed out. A brief account of temperature, atmospheric humidity and rain-fall, and the prevailing growth conditions in some places of Mysore Plateau is given.

Based on the sizes of the successive leaves and internodes the author has recorded four growth periods—or curves—during a year, each one of these being parabolic in nature. Of these the first and the third are larger curves, hence major, while the second and the fourth are smaller in duration and magnitude, hence minor. The differentiation into the four curves is noticed in all branches of perennials with large number of leaves formed during a season. Where a smaller number is formed there is the merging of the first and second, as also the third and fourth, resulting in only two curves during any year. Each curve represents the growth of a season or period forming the 'Growth Parabola'. Since animals also manifest similar features in their activity this may be appropriately styled as the 'Biological Parabola'. The magnitude and duration of the growth-curve depend on the degree of agreement between the prevailing growth conditions and growth of a plant.

The lower plants are as much affected as the higher plants. This has been shown by taking a few representatives from the different groups of plants. In all these members not only vegetative but also reproductive parts manifest the parabolic size variation. The sizes of sporophylls, sporangia and spores are directly related, and best sized structures generally develop about the middle of the seasonal part of a plant bearing these.

INTRODUCTION

The subject of growth happens to be an elaborately studied part in plant physiology. Volumes have been written on this subject and on the development of plant parts under natural and artificial conditions. The various aspects of growth with and without stimulation, have also been studied by many. Extensive work has been done and elaborate literature is available on the growth-rate not only from season to season but also from time to time during a season. Changes in weight, diameter, size and length have been the several aspects which have drawn the attention of the various investigators, and long and short time interval records have been taken to explain this feature. An old reference on this subject happens to be by Sachs (1873) who selected the study of the linear changes in plant parts during the hours of the day. Gregory (1921) has studied the changes in the area of the leaf, while Briggs, Kid and West (1920 a & b) assessed growth in terms of weekly increase in leaf area and dry weight. Friesner's work (1920) on the radicles of *Cucurbita* indicates an attempt at short time intervals recording and this has shed some light on the daily variations and rhythm in the growth of plants. Weight fluctuations in plants from time to time during the hours of the day were recorded by me and published in 1945. During my work on growth and other activities the advantages and efficacy of high magnification and short interval recording have been sufficiently stressed. The high magnification employed has enabled me to record the several activities of plants not only at intervals of

a few minutes but also a few seconds. In all these activities recorded and published by me during 1942-1946, the presence of oscillations in the rate has been explained in detail. I have noticed these oscillations manifesting themselves at intervals of a few minutes or even less than a minute. Just as in other activities, in growth also, oscillatory variation in rate is noticed, even when the external conditions are constant. For the work on growth young roots, stem, leaf and flowers buds were selected and the recording was done with these parts in tact on the plants. Probably the changing Ph of the plant body, oscillatory changes in the water-content of the plant (Krishna Iyengar, 1946*a* & *b*) and the attendant stretching and contraction of the tissue (Krishna Iyengar 1946*c*) must have in no small measure contributed to the oscillations in the rate of linear changes during growth. A diurnal variation in the diameter of stems of several plants has been reported by Mac Dougal and others (1924) and it should not come to one as a surprise if changing water content of the plant body is also responsible for this feature. The records of Went (1925) and Silberschmidt (1926) show oscillations in the growth rate, but these have escaped the notice of these investigators.

Most of my observations of the several activities of plants were of short or long duration with constant external conditions during the period of observation. These have revealed the existence of oscillations in the rates of these activities, and growth which happens to be the result of the harmonious adjustment to the several factors shows, by its oscillatory variations, that there should be similar variations in the harmonic and enzymatic activity and the resulting tone of the living matter from time to time.

While studying growth, one's attention is naturally drawn to the effect of seasons and their influence, and the way Life has adapted itself to these forces for its survival, growth and expression. Extensive literature is available to explain the influence of seasons and their succession on the pattern of plant's life and structure (Schimper 1891, McGregor 1932). The significant contrast in the appearance of a plant during the different season is invariably a reflection of the contrast in the nature of seasons and the resulting variation in the growth conditions. This is quite striking in places with a pronounced seasonal contrast. The rhythmic succession of seasons and changing weather conditions are often associated with the oscillatory variations in the sunspots number (Stetson 1937), and these are expressed by changes in the structure of a plant denoting the plant's constant effort towards an efficient adjustment to the changing conditions. The addition of an appreciable number of large leaves and the high rate of growth and other activities during spring, and a highly reduced activity during winter, form an interesting expression of the seasonal changes and their contrast and these have their counterpart in the internal structure, thus making the plant body an efficient record of weather conditions and their changes. Investigators like Douglas (1919) and others have taken advantage of this feature to study and explain the climatology of places through ages.

While growth changes are oscillatory and generally follow a definite annual rhythm, I have noticed parabolic variation in the sizes of successive leaves, internodes and axillary branches formed even during a season, suggesting the existence of a rhythm even here. A paper on this was communicated to the session of the Indian Science Congress of 1946. Further work done by me in this field has revealed a similar variation in the successive flowers and fruits as also seeds formed during a season or even part of a season denoting the varying vigour of the different parts. A critical study of the development of the individual parts has revealed a similar variation in the vigour of the different regions of any part. All these points have been discussed though briefly in my paper published in 1947. My recent study of the development of the underground parts has shown that these are as much affected as the aerial parts and also manifest similar variation in size and vigour.

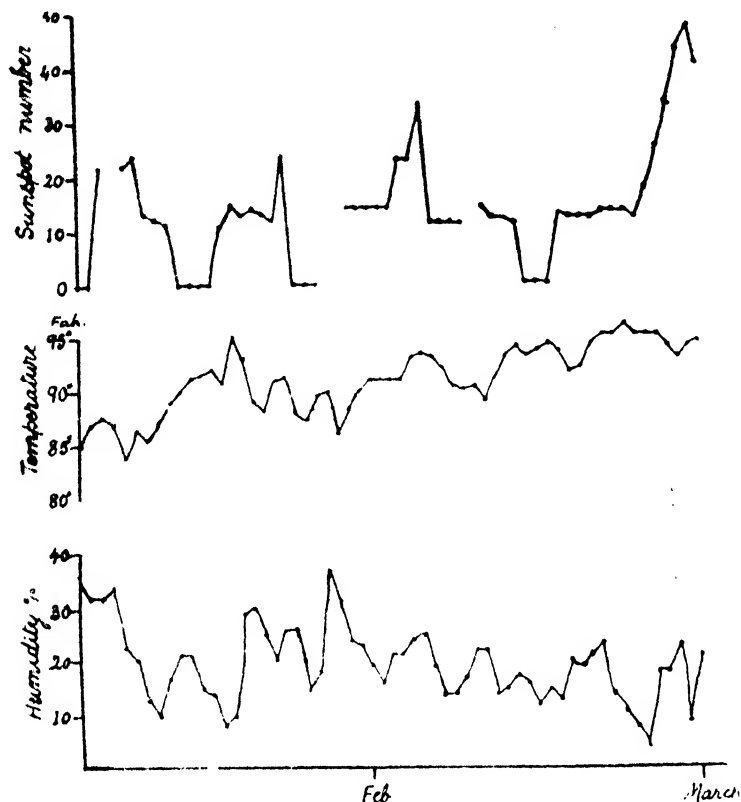
In my previous work (Krishna Iyengar 1947) I have explained that the parabolic variation noticed in the sizes of successive vegetative and reproductive parts of a plant denoted a similar variation in the vigour of these parts also. The preliminary work connected with the propagation of plants, making use of this feature, has also been explained in the paper mentioned above. It has been pointed out that this parabolic growth and vigour variation seems to be a feature of not only plants but also animals. A brief reference has been made in the paper stressing the importance of this knowledge in plant and animal breeding grafting, propagation and cultivation of plants. At the first instance this biological principle was applied to the cultivation of sugarcane, cotton and paddy. The work of four years convinced me that there are tremendous economic possibilities, since the application meant a significant increase in yield per plant under the existing conditions. This has been published in 1951 in the form of a note. In fact this feature could be exploited for the propagation and cultivation of almost all crop plants and fruit plants. In Horticulture, Sericulture, Sylviculture and animal rearing also this knowledge seems to be of some significance. Elaborate study and data collected by me from 1945 till now have denoted the scientific and economic importance of this feature. I am inclined to feel that this parabolic variation in Life—or 'Biological Parabola'—is one of the secrets of Nature one has to exploit for the economic betterment of Mankind. The urgent need for its exploitation to ensure better crops and better plants, and the significant trends and possibilities noticed in this direction, are primarily responsible for this present work.

SEASONAL CYCLE AND GROWTH CONDITIONS

Since a plant's growth is the result of its response to several factors any variation in these will necessarily result in a change in the growth rate. It is well known that the clearly defined growth-rings are associated with the sharp contrast in seasons—so very characteristic of the temperate areas. The striking contrast in the plant's activity due to seasonal changes has left an indelible impression on the external as well as the internal structure of a plant. In tropics this contrast is generally wanting, and with this feature the structural contrast is not likely to be so marked. In general, the plants of any area follow a certain course of development in conformity with the climatic conditions of the place, and the latter will be changing from place to place. The present observations are mostly from areas round about Mysore and Bangalore belonging to the Mysore plateau, with growth conditions not unfavourable during any part of the year since the extremes in temperature are absent. Thus, the summer months are not too hot and the winter is not too severe either, the maximum temperature during the year ranging from 70 to 95 degrees Fah. and occasionally, though for a day or two, touching 100 degrees. No doubt humidity will be changing in a significant way since some months are without any rains, hence are likely to be very dry with the lowest percentage of atmospheric humidity.

The present work is connected with plants mostly under field conditions and as such an enquiry into the weather conditions prevailing at the place seems to be necessary to understand the effect of these several factors. Since this work was started during the early months of 1945 data were collected for temperature, humidity, rainfall and sunspot number for the months of February, March and some of the succeeding months of the year 1945, and a chart has been drawn up. Fig. 1 shows the graphs of these based on the daily readings. This work was undertaken to understand the relationship among the several factors as an aid to determine the growth conditions during different months from February since most of the trees started sprouting during early part of February. A few started their activity even during the third or fourth week of January. The variations in the sunspot number and temperature appear to show a general agreement and the

high temperature preceding the advent of rains will be preceded in its turn by an increase in the sunspot number. Highly reduced atmospheric humidity, increasing sunspot number and temperature and very low water-content of the soil are the characteristic features of the months of February and March.



TEXT-FIG. 1.

Graphs showing the daily humidity, temperature and Sunspot number during February and March 1945.

The influence of sunspots on weather and growth of plants is sufficiently stressed by Douglass (1919). The general agreement between the sunspot number and plant's growth through ages has been noticed and recorded by him. According to him the growth rings are of great meteorological significance, and very useful to explain the changes in weather from year to year. But from his recorded data the seasonal and intra-seasonal variations cannot be understood clearly nor followed closely. My observations published in 1947 have shown and proved that the external structure of a plant can explain changes in growth conditions not only from season to season but also from time to time during a season. From the observations of Douglass it is clear that there is a close agreement between the sunspot maxima and peaks in the sizes of growth rings and that the major and minor peaks in the maxima occur at intervals of about 23 and 11.4 years respectively. Less prominent peaks occur at intervals of even a few years. The data collected by me show an oscillatory variation even at intervals of a few days. Since the sunspot number and ultra-violet radiation are directly related (Stetson 1937), it may be inferred that significant variation in the ultra-violet constituent of light seems to be a possibility, if one can judge from the changing number from day to

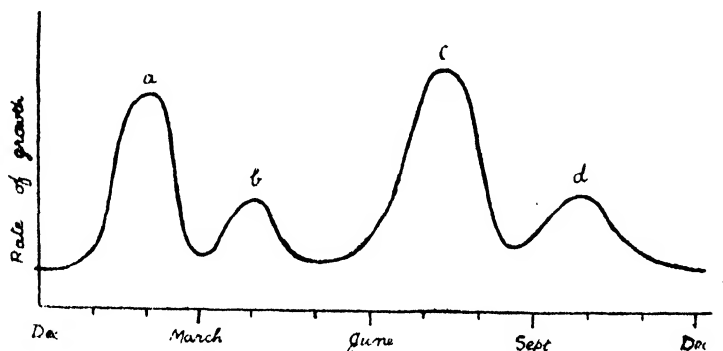
day. At times a daily variation of 20–30 per cent seems to be a possibility according to Stetson. In view of the above it will not be surprising if the growth-rate is affected even at short intervals thus making the structure of a plant an efficient record of the weather changes at short or long intervals.

The dry months of February and March are succeeded by April and May when there are the occasional showers preceded by thunder and lightning. These months are warm and the humidity is not low either. The increasing moisture of the soil will be favourable for a good rate of growth in plants. The South-West monsoon sets in by about the middle of June and the rains are accompanied by heavy wind, and cooler days succeed the warm summer months. These conditions prevail till about the beginning of September, by which time the South-west monsoon also comes to a close. After a brief lull for a few days the North-east monsoon sets in during the later part of September, and generally continues till about the end of November. Heavy rains of short duration and warm days are quite characteristic of this period. Generally from about the end of November the days are clear and get cooler as days pass by. On account of the changing position of the sun the days become shorter, and this trend is maintained till about the 13th or the 14th of January. From this time onwards the days get longer and the temperature also takes a gradual upward trend. Till about the last week of March the days are clear and the earliest rains for the year will be generally during this last week, or during the first week of April. In fact during 1945 the first rains were only on the 31st of March.

It is strange that just at the time of the year when the water-content of the soil is almost at its lowest, many of the trees start sprouting. This will be during the end of January or during the early part of February and the peak in the growth rate is reached by about the end of February or a few days later. The pot plants as well as those in the field have shown the same feature. The growth activity which had reached the lowest rate during December and partly January manifests itself visibly and the deciduous trees which had shed their leaves for a short period of rest, start their first flush for the year. That there is increasing rate of growth as also rich foliage added during the succeeding weeks when the weather is warm and dry and the moisture in the soil is lowest, is a feature which needs clarification. Rest in all the woody plants is accompanied by certain changes in cell-contents (Samish, 1954). Accumulation of food reserves has been a feature preceding dormancy or rest in not only the plant parts but also the plants as a whole and even protoplasm undergoes alteration. All these processes denote a partial dehydrolysis of the cells. With the break in rest, reverse processes start manifesting themselves, the rise in external temperature aiding not only in breaking the rest but also in the several processes. It is quite possible that the plant reserves are converted into mobile products before sprouting and that these probably introduce not only a high rate of respiration but also a high osmotic pressure to serve as a mechanism for an efficient absorption of water from the soil in spite of its poor moisture-content. Instances are many to show the high degree of adaptability of plants in their osmotic adjustment to suit the environment. The investigations of Ursprung and Blum (1921) have shed considerable light on this point. With an efficient adjustment of the plant the rate of growth increases and the peak in the rate is reached by about the end of February. Even the pot plants regularly watered behave similarly signifying the existence of a common factor affecting the rate of growth in both, favourably or unfavourably.

Thus with the advent of a period with favourable conditions there is the spurt of growth. During a year four spurts are generally noticed. These spurts—or growth periods, are represented in the form of a graph (Fig. 2) where each period is shown as a curve denoting the varying rates of growth from time to time during the period. These growth curves are slightly smoothened in the figure. For the proper understanding of this aspect hundreds of plants had to be carefully observed

and their development watched from time to time. The graph is based on the growth noticed in the plants of many parts of Mysore State. With slight modifications this may hold good for the plants in other parts in and outside India. This graph is based on the lengths of the successive internodes and sizes of the successive leaves formed from time to time during a year. Of the four curves the first one is noticed to be present during the months of February and March. Since early part of February happens to be a sprouting period for most of the plants this curve (a) of the graph is introduced to illustrate the feature. The lengths of the successive internodes are also represented in the figure and the varying sizes of these convey an idea of the varying vigour from time to time. The duration for this curve happens to be nearly two months, the peak in rate being noticed by about the end of February. Towards the end of March depression in the rate sets in, as seen clearly in the shorter internodes and smaller leaves. Since the two months are



TEXT-FIG. 2.

Growth curves of a year. Explanation in the text.

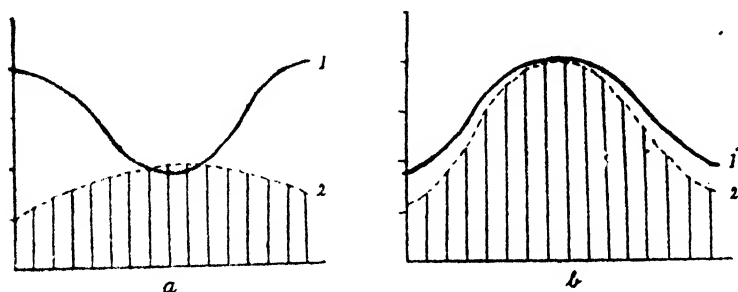
dry months this growth curve has its own interesting aspects. It is noticed that the peak in rate is reached at a time when the days are dry and warm and the moisture content of the soil is very low. The depression in the rate of activity noticed about the end of March and preceding the summer showers cannot be ascribed to the further depletion of moisture in the soil, since even regularly watered plants in the pots manifested a similar feature. This common feature can only be explained as due to a rhythm quite in harmony with the seasonal rhythm. After a short period of depression in the rate there is a second spurt in the growth. This starts during the early part of April after the early summer showers set in, and ends by about the middle of May as represented by the curve (b) of the graph. The duration of this period is shorter and the magnitude is also less although the water-content of the soil is much higher. But the temperature goes on increasing being in the neighbourhood of 95°F or slightly more by the end of April. The second curve is clearly separated from the first and is seen in all plants and their branches sprouting in time and having a large number of leaves added to their branches. As instances may be cited species of *Morus*, *Bignonia* and others. Where there is some delay in sprouting or where the number of leaves added during the period is small, the first and second curves cannot be differentiated. The growth rate is very low from the middle of May for a period of about a month. The temperature during May is usually high, ranging from 95–100 degrees and only on rare occasions during the month touching even 100°, this being noticed only for a day or two. The period of two months April and May is characterised by the summer showers which are often preceded by the thunder storms. Total rainfall during this period including the last week of March will be about 4 or 5 inches or a little more. On account of the heavy electrical charges it may be expected that the rains during this period add lot of Nitrogen to the soil. With

the advent of June the temperature gradually goes down, and with the water-content of the soil not unfavourable the third growth curve (c) makes its appearance. During the second or third week of June the South-west monsoon sets in and the conditions seem to be favourable for a high rate of growth till about the end of August. The heavy wind and the constant showers bring down the temperature, the maximum for these months ranging from 80 to 85 degrees. In fact this period happens to be the longest one for growth noticed during the year, extending from about the middle of June till the beginning of September. The total rainfall during this period will be about 12-13 inches. Average growth rate is high, and the largest number of leaves and internodes are added on during this season. Even the size of the leaves is appreciable. By about the beginning of September the growth rate shows a downward trend or depression, and this will be present for about 15-20 days. During the later part of September the North-east monsoon sets in. The heavy wind so very characteristic of the South-west monsoon is absent during the second monsoon. The temperature also goes up. Bright and sharp sun, and heavy showers in the afternoons or in the evenings characterise the second monsoon period. While the first monsoon is a season of very heavy rains in the Western Ghats area of Mysore, and moderate showers in the maidan parts of the State, during the North-east monsoon the maidan parts get more rains. At times this rainy season may extend up to the end of November. During this period the fourth growth curve (d) for the year manifests itself. The positions of the second and fourth curves seem to be almost similar. These in fact succeed the first and the third which are periods of high rate of growth and the pause after these seems to be short. Just as in the previous case the smaller number of leaves formed as in some of the plants or slight delay in the response of the plants or their parts to the growth-conditions may result in the absence of the sharp distinction between the third and the fourth curves. Thus in all plants the first and the third are well differentiated, while the second and the fourth may or may not be. If all the curves are distinct there will be four curves, while there will be only two big curves when the second and the fourth are not clearly separated from the preceding ones. It may thus be stated that the first and the third curves stand out clearly. Even the growth rate during these two seasons seems to be more than the same during the other two periods. We may say that the first and the third may be taken as the major growth curves while the other two as the minor ones. Countless number of perennials can be cited as example for this feature. This situation in the annual cycle reminds one of a similar picture in the daily cycle. Although usually two periods of high growth rate are reported as the general daily feature, there are in some, according to Friesner (1920), 2-4 peaks in the daily growth-rate in plants.

All these growth curves are slightly smoothened for a general representation. While the smooth curve generally holds good for the plant with a low rate of leaf production this is noticed to be composed of series of smaller curves in all plants with a large number of leaves, added during a season. The finer curves or 'oscillations' noticed in the leaf sizes and the lengths of the internodes are noticed in the sunspot number also. This makes one suspect the presence of a relationship between the two.

While the previous general description has dealt with mostly the perennials, it may be stated that even the annuals show the same parabolic growth variation with finer 'oscillations' in the curve. The growth of an annual can be compared to a seasonal branch, and what is noticed in the former is but an image of the latter. These curves may be pronounced or not, depending on the season in which the annual or the seasonal branch starts its growth activity. It has been a common observation that the annuals cultivated during off-season rarely grow well and the belated sprouting of any branch will manifest a similar feature in its subsequent growth, since growth conditions are not quite favourable. The best results are thus

obtained when the period of cultivation and the seasonal growth conditions agree. The following diagrammatic sketches (Fig. 3) will illustrate this point. Even with the best of cultivation one may get different results since seasonal factor will



TEXT-FIG. 3.

Graphs showing the relationship between seasonal conditions (1) and growth of plant-parts (2), without agreement (a) and with agreement (b).

influence the growth conditions in a significant manner. These are seen in (a) and (b) of the figure. This applies not only to the flowering plants but also to the non-flowering.

PARABOLIC VARIATION IN GROWTH

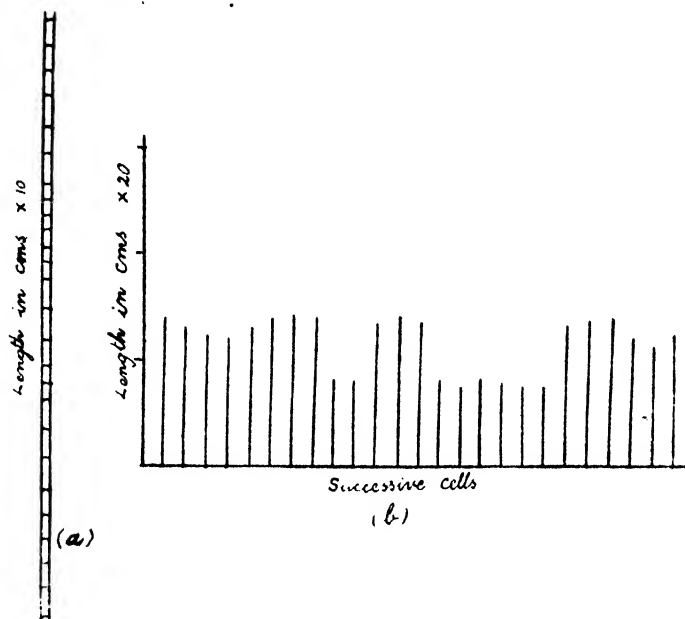
For describing this feature countless number of plants had to be selected and critically studied from time to time. Although most of the plants happen to be Angiosperms a few representative forms from other groups have been selected to understand the working of this growth principle in them. Members like *Spirogyra*, *Chara*, *Sargassum*, *Agaricus*, *Riccia*, *Marchantia*, *Lycopodium*, *Selaginella*, *Ophioglossum*, *Adiantum*, *Asplenium* are some of the cryptogamic plants included in this although many others have also been studied. *Cycas*, *Araucaria* and *Pinus* species are the Gymnosperms selected, while among the Angiosperms the number of plants studied are too many to be listed, but the plants described in this section and others may be taken as the representative forms of the groups.

CRYPTOGAMS AND GYMNOSPERMS

1. *Algae : Spirogyra* : A few filaments have been examined and the lengths of the successive cells have been measured. The following figures (Fig. 4) shows a sketch of the filament (a) and the lengths of its successive cells (b). The details incorporated refer to a filament which was neither too young nor too old. From the figure it can be seen that there is a significant difference between the shorter and the longer cells. Younger filaments show even more pronounced oscillation in size on account of the intercalation of cells with meristematic nature. This is represented in (b) of the figure. Even in an old filament a significant difference is noticed in the lengths of the cells and this difference between the longest and the shortest cells of the filament being about 40 percent or more. The study of the zygotes and their size variation could not be taken up since several other factors are involved in their development and size.

Chara : The lateral branches at the nodes of the older branch were separated and several of these have been studied in connection with not only the lengths of the internodes but also the sizes of oogonia and antheridia as also their distribution on these branches. The diagrammatic sketch (Fig. 5) of a branch, and the data connected with the sizes of the sex organs introduced below will convey an idea of the degree of variation. In all the branches the first internode was the

shortest and the node just above this was devoid of the six organs, hence sterile. Usually the 2nd, 3rd and the 4th nodes, and occasionally the 5th also, bear the sex organs. It is generally noticed that the best sized oogonia and antheridia are borne at the 3rd node. Next in order are the basal ones, while the distal ones, usually the topmost, are the poorest. The last ones are very much reduced in size, and at times missing also. In the older branches it was noticed that only the lower two oogonia had developed into the oospores demonstrating the greater robustness of the lower two. The several nodes above the 4th or the 5th are invariably sterile.



TEXT-FIG. 4.

Diagrammatic representation of *Spirogyra* filament
(a) and lengths of the successive cells (b).



TEXT-FIG. 5.

Chara 'leaf' with oogonia and
antheridia. 1, 2 and 3-order of sizes.

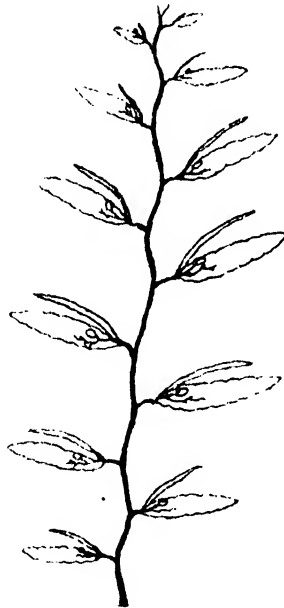
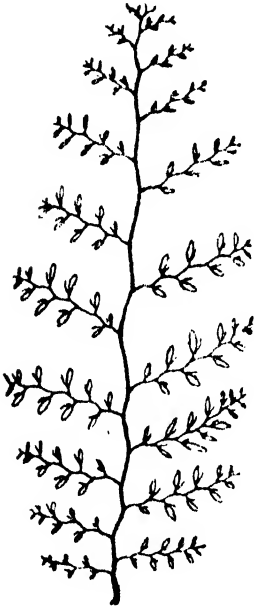
Sargassum : Two diagrammatic sketches of one of the species of *Sargassum* are introduced in (a) and (b) of the Fig. 6. The entire plant is represented in (a). The general outline of the plant reminds one of the outline of a branch (b) of the plant, and this in its turn approximates to the outline of one of its leafy structures, or 'phyllode' (c). The most robust branches seem to be distributed about the middle. The 'phyllodes' are also larger about the middle of the branch and the basal part seems to be next in order of vigour while the distal part appears to be the poorest as is evident from the smaller 'phyllodes' on this part. Even in a single leafy part the middle portion seems to be more vigorous than the ends. In several other algal members, belonging to the Green, Brown Red Algae, a similar feature is met with, and it is unnecessary to describe them in detail since the above description and a casual mention of others should suffice.

2. *Fungi* : *Rhizopus*, *Agaricus* and *Coprinus* are the forms selected from this group though for a brief study. In *Rhizopus* cultures it was noticed that the first formed sporangia were often slightly smaller than those formed subsequently and the last formed ones were also smaller. This knowledge may be useful in the isolation of the sporangia and the spores for a healthy culture of the fungus.

In *Agaricus* and *Coprinus* the gills were separated and the sizes of the spores along the length of the gill were critically studied. It was noticed that the middle of each gill generally bore slightly larger spores than the ends. These were slightly

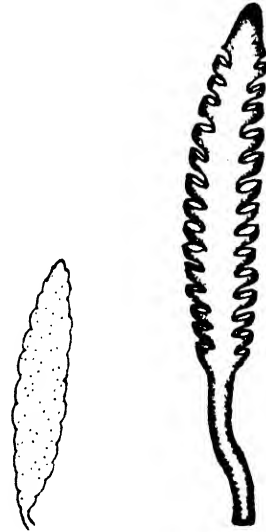
longer and of a larger diameter. With this knowledge of the vigour distribution in the gill it will be easy to isolate the desirable spores so important in the cultivation of edible fungi.

3. *Bryophytes* : A general study of a few representatives like *Riccia*, *Marchantia* and one or two mosses were taken up. Not only external but also internal structure of the forms was thought of, and this was mostly connected with the growth peculiarities. It has been a common observation that the thallus of *Riccia* and *Marchantia* and other liverworts which possess a thick thallus show the thin ends and the thicker middle part. The width is more about the middle and the two ends are slightly narrower. The margin is thinner than the middle part, and shows poorly developed chlorenchyma and parenchyma. *Marchantia* and some others show larger number of 'chimneys' as also better sized ones per unit area about the middle, while those towards the margins are fewer in number and smaller in size. The gametophores of *Bryum* and *Polytrichum* species show the poorly developed basal and the terminal 'leaves' with the more robust and better formed ones about the middle.



TEXT-FIG. 6.

Thallus of *Sargassum* sp., its branch and phylloide.



TEXT-FIG. 7.

Spike of *Ophioglossum* sp.

4. *Pteridophytes* : *Lycopodium*, *Selaginella*, *Equisetum* and *Ophioglossum* species and a few ferns were taken up for study. The first three will be dealt with together since the possession of stem, leaf and strobilus forms a common feature of these. In all the three, any branch will show the first and last formed reduced leaves, the best sized ones being distributed about the middle of the seasonal growth of the branch. In *Equisetum* the scale leaves also show this feature, and the length of the successive internodes of the branch illustrates this point very well. In all the members the basal and distal sporophylls of the strobilus are reduced in size, while those about the middle are larger. Even the sizes of the sporangia borne on the successive sporophylls show the same parabolic variation and the close relationship between the sizes of the sporangia and the sporophylls. A critical examination of the spores from the several sporangia has revealed that the middle

sporangia generally bear larger spores. There seems to be greater uniformity in the sizes of the spores developing from the middle part of the strobilus than from the ends.

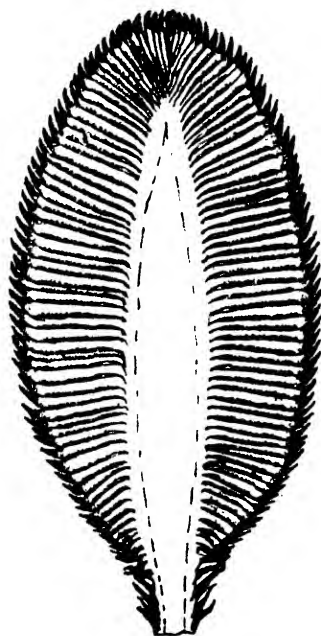
Even in *Ophioglossum* the sporangial size-variation is a significant feature of the spike (Fig. 7). The sporangia are arranged in two rows along the spike, and of these two or three on either side towards the base and more than this number towards the distal end of the spike show a reduced size. These show a high degree of variation in the sizes of the spores, the smaller ones being more numerous. The sporangia about the middle are invariably larger and the spores are more uniform in size as also larger.

A few of the ferns have also been studied. A photograph of *Asplenium* (Fig. 8) is introduced in Plate IV. It is possible to notice in this plant also the features met with the other members. The sizes of fronds show a parabolic variation. The smaller fronds precede and succeed the larger fronds formed during the middle of the season, one kind gradually merging into the other. Even in fertility the fronds about the middle are better than those towards the two ends, the latter showing a gradual decrease in this with the resulting sterile fronds at the two ends. The distribution of fertility in a single frond shows the same feature. A long basal sterile region is succeeded by a longer fertile part and this is followed by short sterile part towards the apex, as is well illustrated by the sporangial distribution along the length of the frond. There is also a transition from the completely sterile marginal portion to the fertile region towards the midrib. The width of the frond as also of the sporangial zone increases gradually from the base towards the middle and sharply tapers towards the apex. Even the density of the sporangia seems to be more in this region and gradually becomes poorer towards the margin and the ends, with the sterile region succeeding this. All these can be clearly seen in the figure.

Adiantum is the next genus selected. On account of the compound nature of the frond and a slight variation in the distribution of the sporangial region this was taken up for a critical study. The highly branching frond of *Adiantum* forms a striking contrast to the simple one of *Asplenium*. Here also the first and the last formed fronds during a season are small and sterile, and between these and the large fertile fronds about the middle, there are those occupying an intermediate position. The middle ones are not only large but also bear the largest number of sporangia, and even these show regions of varying fertility and growth vigour. From the small and sterile leaflets towards the two ends—basal and distal—there is a transition in growth and fertility to the middle part where the leaflets show a highly increased growth and fertility as seen by the larger leaflets with the larger sori and sporangia and a larger number of these. Just as in the previous forms the larger spores and larger sporangia generally go together.

5. *Gymnosperms*: Many members have been observed but only *Cycas*, *Pinus* and *Araucaria* species have been introduced in the descriptions. In *Cycas* the leaves forming the crown show a parabolic size variation, the basal and the distal leaves being a little smaller and showing a transition to the larger ones about the middle of the crown. Even in a leaf the pinnules towards the base and the apex are smaller while those about the middle are larger. A variation of 12–15 per cent or more is noticed not only in length but also in breadth. Even the persistent leaf-bases of a season show the same feature, the middle ones are larger than those on either side. In the male cone one can notice the successive sporophylls showing the same parabolic variation in growth-vigour and fertility as is evident from the reduced and sterile or partly sterile sporophylls towards the base and apex and the well developed ones towards the middle. The diagram introduced below (Fig. 9) will show some of the points mentioned above. The larger sporophylls invariably bear larger sporangia and larger spores. The individual sporophyll shows the sterile distal and basal parts and a fertile middle region which is generally

broader also. In a section of this part it is observed that the larger sporangia are concentrated towards the middle and that the smaller ones are towards the margin. The sizes of the spores also vary proportionately.



TEXT-FIG. 9.

Sectional view of the male cone of *Cycas circinalis*.

The megasporophylls also are similar. In the cluster of sporophylls it is noticed that the first formed ones which are very small and without any ovules, are towards the base. Gradual increase in size and fertility as the middle ones are approached and a steady fall of these in the sporophylls towards the top characterise the cluster. This is denoted by the size and number of ovules. From the sporophylls towards the base and without any ovule there is a gradual appearance of reduced number of smaller ovules on those just above, and these are succeeded by the sporophylls about the middle with larger ovules and a larger number of these on each. There is again a gradual reduction in the number and size of the ovules towards the top. These ovules on the basal and distal sporophylls show a very high percentage of degeneration during the later stages of development. In each sporophyll it is noticed that the basal and distal ends are sterile and only the middle part bears the ovules. Most of the ovules towards the base either develop into very poor seeds or degenerate during later stages.

A few species of *Araucaria* and *Pinus* have been studied and a brief account of these is given below. Fig. 10 is a photo of *Araucaria cookii*. In this plant the lowermost lateral branches are shorter than those above, and even after years this initial difference in growth vigour is maintained. On each lateral branch there are several smaller branches (dwarf branches) arranged in two series, and the latter bear the small leaves. The lowermost as well as the topmost leaves are smaller than those about the middle.

Other species of *Araucaria* are also similar. In *A. bidwilli* the 'dwarf' branches persist for a few years. Any of these old branches will clearly illustrate the effect of seasons, by the varying sizes of the scale leaves formed along the length of the



Photograph of a plant of *Asplenium nidus*.



Fig. 10. Photograph of a plant of *Araucaria coolii*.

Fig. 11. Photograph of the female cone of *Pinus sylvestris*.

branch. The general outline of the plant is conical with the sharply tapering base and a gradually tapering distal end. This holds good for any of the branches arranged in whorls on the main axis. What is noticed in *Araucaria* is noticed in many other conifers also. As examples may be cited the several species of *Cupressus* and *Callitris*.

In the case of *Pinus* an attempt has been made to study the length of the leaf ('Needle') and the sizes of cones and their sporophylls. The stem bears the scale leaves all over. These are smaller and crowded along a part of the length and this is followed by the part with larger scales showing a better spacing. Thus in the annual growth of the stem one will notice the alternating lengths of these denoting higher and lower growth-rates. In the 'needles' formed during a season also the varying growth vigour is noticed. The lower 'needles' showed an average length of 6-7 inches, the middle ones measured $9\frac{1}{2}$ to $10\frac{1}{2}$ inches, while the needles towards the distal end were ranging from 8 to 9 inches in length. The male and female cones of *Pinus* were next taken up. The Photo (Fig. 11) of the female cones is given in Plate V. In both, the parabolic variation in size and fertility is noticed. The small sterile sporophylls towards the base of the cone, transition to the larger and fertile ones towards the middle and a gradual decline in size and fertility of the sporophylls towards the distal end remind one of a similar situation in the other forms already described. The size and fertility of the ovules as also the size of the pollen grains are closely related to the size and vigour of the sporophylls. A general study of the cones of *Araucaria* and others has revealed that these also show similar features.

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CYTOCHEMISTRY OF THE CELL-TYPES IN HYDRA AND THEIR FUNCTIONAL SIGNIFICANCE

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ABSTRACT

Cytochemical techniques have been employed to demonstrate the localisation of alkaline phosphatase, RNA and DNA, and protein in the different cell-types in the body of hydra. The epitheliomuscular cells show a negative reaction for alkaline phosphatase and basophilia except in the nucleolus, but a positive reaction for protein. The nuclei are large and faintly stained. The interstitial cells are rich in RNA and protein but show only traces of alkaline phosphatase. The nuclei are smaller with condensed chromatin. In the cnidoblasts, the nematocysts differ in reactions and the nuclei are poorly reactive. The nerve and sensory cells are also poorly localised. In the endoderm, the secretory gland cells show intense reaction of these substances in the cytoplasm. The nuclear Foulgen reaction is similar to those of the interstitial cells. The nutritive muscular cells have rich alkaline phosphatase and some protein but little basophilia. The nuclear conditions are similar to those of the epitheliomuscular cells. The different regions of the body—tentacle, hypostome, stem and basal disc—have distinct cytochemical patterns. The results are discussed in relation to their functional significance.

INTRODUCTION

In contemporary Biology, application of cytochemical techniques to understand the functional aspects of cellular organisation is becoming increasingly evident. Detection of various cell-substances at sites of their activities yields valuable data concerning their role in the vital processes. At the same time, such findings also provide an index of the function of a cell in relation to others at the various levels of activity. The body of hydra is composed of only seven types of cells which carry out all vital functions. Further, hydra shows an almost unlimited power of regeneration and growth. Kedrowski (1941), Brien (1942) and lately, Tardent (1954) have found a rich deposition of ribonucleic acid in the interstitial cells. In a preliminary communication (Sanyal and Mookerjee, 1956), the two cell-layers, viz., ectoderm and endoderm, have been reported to possess markedly different concentrations of certain substances including RNA. A cytochemical study of the different cell-types in the body of hydra is now undertaken to understand their functional significance. The localisation of the enzyme, alkaline phosphatase, the nucleic acids (RNA and DNA) and protein is followed.

MATERIAL AND METHODS

Hydra vulgaris Pallas (phase *orientalis* Annandale, 1911) was used in this investigation. Specimens were collected from local ponds and grown in the laboratory. They were cultured in glass aquaria containing tap water and a few aquatic plants and were fed with daphnia. Prior to fixation, they were starved for 24 hours.

Hydras tend to become rounded on fixation. This could be prevented by suddenly showering a jet of fixative on the animals from a pipette. They were dehydrated in alcohol and embedded in paraffin. Sections of 5μ to 8μ thickness were made. Gomori's technique (Gomori, 1952) was used for the detection of alkaline phosphatase, with chilled 80 per cent alcohol as fixative and sodium glycerophosphate as the substrate. Sections were incubated at 37°C for 1-3 hours

at pH 9.4. For study of distribution of RNA, material was fixed in Zenker's fluid containing 5 per cent acetic acid and stained in 0.1 per cent toluidine blue in 1 per cent alcohol. The Feulgen technique (after Glick, 1949) was followed for DNA. Carnoy's fixative was used and hydrolysis in 1N HCl for 7 minutes was found most suitable. For proteins, the Hg-BPB reaction described by Mazia *et al.* (1953) was employed. Fixation was done in alcoholic Bouin's fluid. An alcoholic solution of 0.1 per cent bromophenol blue containing 10 per cent mercuric chloride was used. All the preparations were mounted in canada balsam.

RESULTS

1. *Alkaline phosphatase in the cell-types of hydra :*

Localisation of alkaline phosphatase (Fig. 1) presents a sharp contrast between ectoderm and endoderm cells. The ectoderm cells with the exception of certain nematocysts, are less reactive, whereas the endoderm cells are the most positive ones.

In the epitheliomuscular cell, the nucleolus is the centre of highest activity, and the nucleus shows only traces of the enzyme, but the cytoplasm is negative. The interstitial cells show a moderate reaction. Here again, the nucleolus is the centre of highest concentration. The cytoplasm is very faintly positive. The different types of nematocysts differ in their phosphatase content. The smaller nematocysts, belonging to the volvent and glutinant varieties, which are more numerous on the tentacles, show the highest reaction; penetrants, also common on the body, are negative. In some cases, penetrants are also found to show a positive reaction. Discharged and undischarged nematocysts of the same type are also found to show a similar reaction pattern. The nerve and sensory cells are similar to interstitial cells.

In comparison, endoderm cells show much increased phosphatase content. All endoderm cells are uniformly positive. The maximum concentration in the cytoplasm is found on the free border lining the coelenteron. The secretory gland cells have a denser and slightly more positive cytoplasm. The nuclei of endodermal cells are more positive in comparison with their ectodermal counterparts. The nucleolus is dense and surrounded by a positive nucleoplasm. The mesoglea is thin and negative.

2. *Basophilia in the cell-types of hydra :*

Basophilia varies sharply between different cells of both layers (Fig. 2). In the epitheliomuscular cell, basophilia is restricted to nucleolus, the nucleus is non-reactive and granular in appearance, but the cytoplasm is absolutely negative. The basophilia is most intense in the interstitial cells. The cytoplasm, which is a small area round the nucleus, is most positive. The nuclei are smaller in size but similar to those of the epitheliomuscular cells, in basophilic content. Corresponding to the different sizes of interstitial cells, the stainability also varies. Some of them show a weaker reaction. Different types of nematocysts, again, differ in their basophilia. The volvents and glutinants with short and uncoiled threads show violet metachromasy, while glutinants with long and much coiled threads are negative. The penetrants are mostly negative, but some of them show a positive reaction. There is no reaction difference between discharged and undischarged nematocysts. The differentiation of the cnidoblast is full of interest. The process is initiated by the appearance of a clear area within the cytoplasm. This area gradually increases in size and pushes the nucleus to one side. The nucleus ultimately becomes sickle shaped and loses the characteristic basophilic reaction and shows a faint granular appearance and the nucleolus disappears. Nerve and sensory cells are smaller in size and have a moderate reaction. The nuclei of the

nerve cells are granular and the nucleoli are not visible. The sensory cells are similar to interstitial cells.

In the nutritive muscular cells the nuclear reaction is similar to the epithelial cells. The ground cytoplasm is also generally negative but a number of faintly stained large round granules are sometimes seen. The secretory gland cells are the most positive ones among the endodermal cells. These are smaller cells, found in between the nutritive cells. In sections they generally appear ovoid, the cytoplasmic part of the cell facing the coelenteron being filled with large round particles which give a negative reaction. The cytoplasmic part facing the mesoglea shows no such particles, but a highly basophilic reaction. The nucleus is situated in this basophilic part of the cell. The mesogleal wall shows a negative reaction.

3. *Feulgen reaction in the cell-types of hydra :*

Feulgen reaction is limited to cell-nuclei (Fig. 3). Nuclei of epitheliomuscular cells show uniformly moderate reaction. The nucleolus is prominently seen. The nuclear volume is greater than that of other ectodermal cells. In the interstitial cells, nuclei have smaller volume, and the intensity of reaction is greater. The appearance is granular and in contrast to that of epithelial cells, the chromatin material is condensed. The nucleolus is not discernible. A number of nuclei have been observed in different stages of mitosis. In the cnidoblast the nucleus is attached to the base of the nematocyst capsule, and often appears sickle shaped. Like the interstitial cells, the nuclear volume is less and the intensity of the reaction is greater. Nucleolus is not visible. There is no mitosis.

Nuclei of the nutritive muscular cells are exactly similar to those of the epitheliomuscular cells. The nuclei of the secretory gland cells are similar to those of the interstitial cells. Mitosis is rare in the endoderm cells.

4. *Hg-BPB reactions in the cell-types of hydra :*

The Hg-BPB reaction results in a blue stain indicative of protein localisation. All the cells show good reaction, but the intensity is not same for all types of cells (Fig. 4.).

The epitheliomuscular cells, which have not been found to show much of basophilia or alkaline phosphatase, show moderate reaction for protein in the cytoplasm. The reaction is not uniform but strands of stained material are recognisable in the cytoplasm. In the nucleus, the nucleolus shows intense reaction. There is a clear area in the nucleolus. In the interstitial cells, the cytoplasm shows a uniformly positive and intense reaction. The reaction of the nucleus is similar to that of epitheliomuscular cells. The cnidoblasts show a moderate reaction, the nematocyst capsules, the nuclei and the cytoplasm showing faint stain. The nerve and sensory cells also show faint reaction.

In the endoderm, the nutritive muscular cells are similar to epitheliomuscular cells of ectoderm, but show a larger cytoplasmic area. The secretory gland cells have uniformly stained granular cytoplasm. As in basophilic preparation a large number of round bodies are sometimes seen in the endoderm which gives a very strong reaction. Mesogleal wall shows a highly positive reaction.

5. *Histochemical difference at the various regions of hydra :*

Apart from the cell-types varying remarkably in their cytochemical make-up, the different regions of the body—tentacle, hypostome, stem, stalk, and basal disc—show certain distinctive features in their histochemical patterns. These regions are formed by the participation of different types of cells in varying proportion (Mookerjee and Sanyal, *in the press*) and often contain certain specialised cells.

Tentacle—Tentacular ectoderm is formed largely by the volvent and glutinant nematocysts and a few slender epitheliomuscular cells. It is highly positive for

alkaline phosphatase and shows violet metachromasy of basophilia and a faint reaction of proteins. The nuclei are pycnotic in appearance. Endoderm is formed solely by nutritive muscular cells which are of smaller size and highly vacuolated nature resulting in weaker reaction for all the tests.

Hypostome.—The ectoderm is formed by epitheliomuscular cells, cnidoblasts and a fair number of nerve cells. The region gives a weak histochemical reaction for alkaline phosphatase and RNA but moderate reaction for protein. The endoderm is highly interesting. The endodermal wall is thrown into a number of folds, the free surface of which (cells guarding the inner side of the mouth opening) is formed by a row of specialized gland cells. This innermost layer in the hypostomal endoderm is highly positive for alkaline phosphatase, RNA and protein. In fact, it is the region of highest cytochemical activity in the body of the hydra. The inner portion of the folds, formed largely by nutritive muscular cells, appears vacuolated and shows only a moderate localisation of these substances.

Upper region of the body.—The ectoderm abounds in interstitial cells. The ectoderm shows a weak reaction for alkaline phosphatase but intense basophilia. The endoderm is rich in alkaline phosphatase and shows a moderate basophilia. Protein is present in about the same quantity in both the layers.

Stalk.—Cytochemical activity in the ectoderm of stalk is weaker, due to the scarcity of interstitial cells; otherwise this region resembles the rest of the animal in essential points. Endoderm cells are vacuolated like those in the tentacles and secretory cells are fewer resulting in weaker histochemical reactions.

Basal disc.—The ectoderm is formed only of specialised muscle cells and nerve cells which form a flat surface for attachment. The cytoplasm of the muscle cells shows a fibrous appearance. It gives a diffuse and moderate reaction for alkaline phosphatase and basophilia but the cells are rich in protein. Endoderm adjacent to basal disc shows a greater substance localisation than the neighbouring endoderm of the stalk.

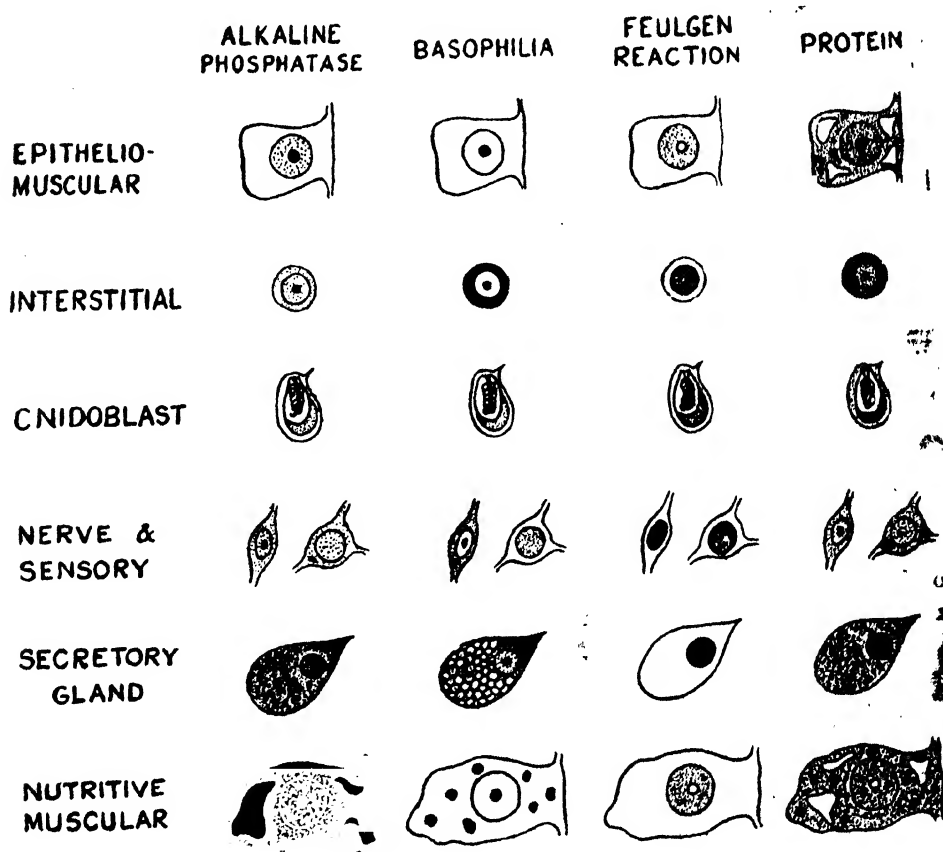
DISCUSSION

The results of this study of substance localisation in hydra show that the different cell-types vary in their cytochemical make-up. Text-Fig. 1 presents a comparative account of localisation reaction in the different cell-types. Most active are the interstitial cells of ectoderm and the secretory gland cells of endoderm. The former comprise the most numerous of the cell-types in hydra (Mookerjee and Sanyal, *in the press*) and the intensity of substance localisation in them, specially that of RNA, is suggestive of great synthetic activity. In view of their well-known proliferative nature, and the not infrequent mitotic figures, the interstitial cells appear to be the sites of intense protein synthesis. This is also evident from the Hg-BPB reaction (after Mazia *et al.*, 1953) signifying protein localization. Brachet (1957) has raised doubts about bromophenol blue as a cytochemical reagent, as the reaction is greatly modified by ribonuclease digestion. In the present work, it has been observed that regions apparently devoid of RNA, e.g., cytoplasm of the epitheliomuscular cell, give positive reaction in bromophenol blue. Although this is not conclusive evidence, it seems reasonable to assume that regions stained by this dye contain some amount of protein. The technique has been specially useful for the epitheliomuscular cells, as these cells have not given positive reaction in other tests. The secretory gland cells are the chief sites of RNA among the endoderm cells. This is obviously related to their secretory function.

It is seen that the interstitial cells have little alkaline phosphatase, but it is to be remembered that in recent studies (Vorbrodt, 1958) association of alkaline phosphatase with protein synthesis has been seriously questioned.

The interstitial cells in hydra have been repeatedly regarded as totipotent (Strelin 1928, Brien and Reniers, 1950, etc.). In the present investigation no

indication is obtained as to their transformation into other cells except into cnidoblasts. Transitory stages in cnidoblast differentiation could be easily identified and the concomitant cytochemical changes could also be observed. Of particular interest is the gradual loss of basophilia during cnidoblast differentiation. Changes in alkaline phosphatase during the cnidoblast formation could not be followed as most of the structural details are lost after fixation. However, it is seen that there is no significant change in the early stages of the process, although later nematocysts are seen to differ in their alkaline phosphatase content. The structural changes during the formation of different types of nematocysts of hydra have been recently studied by Chapman and Tilney (1959 *a, b*) with the electron microscope. Whatever be the biochemical factors involved in their differentiation, there is one common denominator, i.e., loss of cytoplasmic basophilia. A similar loss is expected to be encountered in case of transformation to other ectodermal cells, as basophilia in other cells is always lesser. It has already been seen that there occurs a loss of basophilia during bud formation and gametogenesis (see Brachet, 1950, p. 440). Indications are also available that ribonucleoprotein particles of the cytoplasm in interstitial cells are involved in synthesis of a protein-rich cell product during differentiation of nematocysts (Slautterback and Fawcett, 1959). From all these, it appears not unlikely that differentiation of interstitial cells is related to nucleic



TEXT-FIG. 1.

Diagrammatic representation of comparative cytochemical localizations in the different cell-types of hydra. Reactions within nematocysts have not been shown.

acid synthesis. Support for this idea is available from the observations of Mookerjee and Bose (*in the press*) where increased gonad differentiation was obtained in hydra after exposure to thymus nucleic acid, whereas sodium nucleate from yeast was ineffective.

Absence of detectable amounts of RNA and alkaline phosphatase in the cytoplasm of epitheliomuscular cells and the RNA in nutritive muscular cells poses a problem for their maintenance. At the same time these cells are only rarely seen in mitotic stages. The nuclear aspects of the different cells are being investigated by Mookerjee and Nagarajan (*in preparation*).

The histochemical pattern in the different regions of the body of hydra is understood to be functionally significant. The most active region is found to be the hypostome endoderm which, according to McConnel (1929), undertakes the acid-phase of food digestion. But how far this is related to the high metabolic activity ascribed to this region in the axial gradient concept (Child 1941, for review) is not known. The upper two-thirds of the stem also regarded as stomach (Hyman, 1940), undertakes digestion and assimilation of food and shows greater intensity of substance-localisation. Recently Nair and Sane (1958) have confirmed our previous observation (Sanyal and Mookerjee, 1956) that the endoderm is highly positive in alkaline phosphatase and they have related it to absorption of glucose. It is seen that the endoderm is immediately related to the functions of alimentation, and the protein containing basophilic bodies observed on some occasions in the endoderm cells can bear relation to the state of nutrition of hydra at the time of fixation.

The endoderm cells in the tentacle and the stalk are vacuolated and consequently show decreased cytochemical activity. But the basal disc, which forms the organ of attachment, shows a comparatively higher localisation reaction in both the ectoderm and endoderm in comparison to the stalk. Kepner and Miller (1928) have distinguished the endoderm adjacent to the basal disc as a distinct histological region. Cytochemical findings suggest that these are involved in the secretory processes of these cells. Accumulation of RNA in this region points to the protein nature of the secretion.

Finally, the regionality of cytochemical localisations in tentacle, hypostome, stem and basal disc seems to lend support to the concept of these regions as different individuation fields within the body of a hydra, as has been recently put forward (Mookerjee and Sanyal, *in the press*).

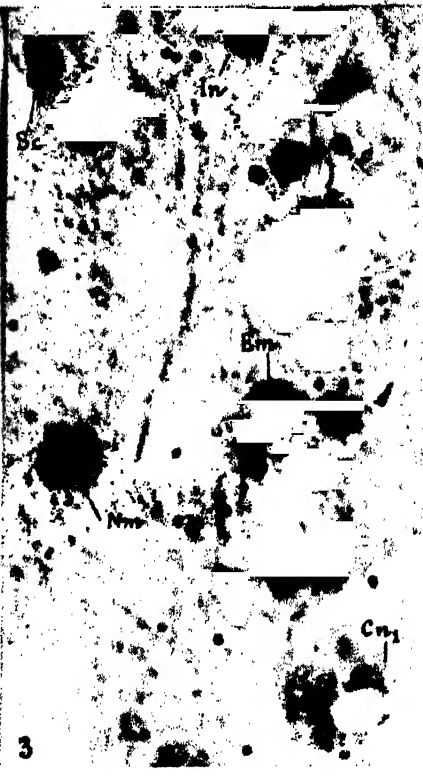
ACKNOWLEDGEMENTS

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EXPLANATION OF PLATE VI

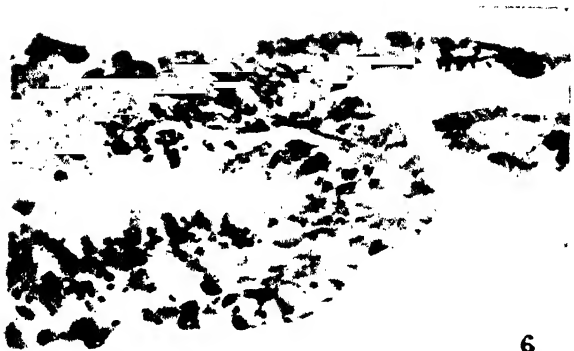
Photomicrographs showing cytochemical localisations in the cell-types of hydra—

- Fig. 1. L.S. of hydra showing distribution of alkaline phosphatase. The reaction is intense in endoderm cells. ($\times 900$).
 - Fig. 2. L.S. of hydra showing distribution of RNA. The interstitial and secretory gland cells are most positive. ($\times 1100$).
 - Fig. 3. L.S. of hydra showing distribution of DNA. Smaller nuclei of interstitial and secretory gland cells show more intense localisation than the larger nuclei of epitheliomuscular and nutritive muscular cells. ($\times 1500$).
 - Fig. 4. L.S. of hydra showing distribution of protein. The interstitial cells are most reactive. The epitheliomuscular cell shows strands of stained area within it. ($\times 900$).
- (Em—Epitheliomuscular cell, In—Interstitial cell, Cn₁—Penetrant nematocyst, Cn₂—Glutinant nematocyst, Sg—Secretory gland cell, Nm—Nutritive muscular cell).





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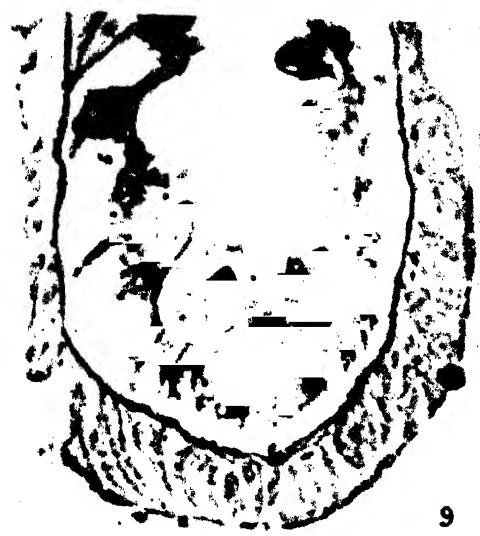
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EXPLANATION OF PLATE VII

Photomicrographs showing distribution of Alkaline phosphatase and RNA in the different regions of hydra.—

- Fig. 5. Alkaline phosphatase in the hypostome and tentacle region, note maximum concentration in the bordering endoderm. L.S. ($\times 200$).
- Fig. 6. RNA in the hypostome and tentacle region. The ectoderm is poorly localised, the endoderm is rich in RNA. L.S. ($\times 340$).
- Fig. 7. Alkaline phosphatase in the stem region. Localisation is more intense in endoderm. T.S. ($\times 260$).
- Fig. 8. RNA in the stem region. RNA is localised in the interstitial and secretory gland cells. The ectoderm shows a greater concentration. T.S. ($\times 340$).
- Fig. 9. Alkaline phosphatase in the stalk and basal disc region. Note fibrous nature of the ectoderm. The adjoining endoderm is highly positive. L.S. ($\times 390$).
- Fig. 10. RNA in the stalk and basal disc region. The ectoderm in the basal disc is most positive. L.S. ($\times 340$).

FURTHER STUDIES ON THE ALIMENTARY TRACT OF THE MILK-FISH *CHANOS* IN RELATION TO ITS FOOD AND FEEDING HABITS

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ABSTRACT

In the alimentary tract of the fish *Chanos*, a pair of complicated pharyngeal pockets, the spiral mucosal folds in the oesophagus, a spacious corpus with giant mucous cells and massive gastric glands and a highly muscular triturating pylorus are peculiar features. They are special adaptations to a diet consisting of algae and molluscs mixed with mud and sand particles. Analysis of the gut contents of different age groups of the genus *Chanos*, points out that feeding habits change from fry, through juvenile to adult, and that the adult fish is not exclusively a plankton feeder.

INTRODUCTION

The morphology and histology of the alimentary canal of teleostean fishes have attracted the attention of many workers. Reviews of the various investigations on the subject have appeared from time to time and therefore, it may be necessary here to confine to the observations published on the genus *Chanos*.

Chacko (1945) made a brief note on the food and alimentary canal of *Chanos chanos* (Forsk.) which he (1949 and 1956) followed up by a detailed list of the food items present in the gut. Kapoor (1954) has given an account of the supra-pharyngeal organs of this fish and has pointed out that it is more related to the digestive than to the respiratory system. Chandy (1956) described in detail the spiral mucosal folds of the oesophagus. Tampy (1958), has critically analysed the food of *Chanos* in three arbitrary age groups, viz., (1) fries and fingerlings, (2) juveniles and (3) adults. His analysis points out, as earlier suggested by Hiatt (1944), that there are possible changes in the food habits of these fishes, accompanying age and growth.

While studying the oesophagus of *Chanos* the senior author observed that the stomach presented certain peculiarities which needed investigation. Further as the accounts of the various organs are scattered in the literature, it is necessary to make a consolidated account of the whole alimentary canal and review the existing knowledge in relation to the feeding habits of this commercially important food fish.

MATERIAL AND METHODS

Specimens of adult *Chanos* fixed in Bouin's fluid were obtained from Kottayam Biological Supplies. With a view to tracing the course of the food-particles, fries specially fed on Carmine, were obtained from Mr. P. I. Chacko, Director, Madras Fisheries. For histological studies, sections were cut at 6-8 μ and were stained with Delafield's Haematoxylin and counter-stained with alcoholic Eosin. Mallory's Triple stain and Hiedenhains Haematoxylin were also used as alternative stains. Meyer's Mucicarmine was used for the detection of mucous cells.

ECOLOGICAL CONSIDERATIONS

Chanos chanos is a large Clupeoid fish of the family Chanidae having a wide distribution in the Indo-Pacific Ocean, from Red Sea to Hawaii. It is thus an essentially marine form; but it invades the inshore waters for breeding. Incidentally it may be mentioned that *Chanos* gets acclimatized to fresh water and can be cultured in ponds. Chidambaram and Unnie (1946) have shown that the maximum growth of *Chanos* is in freshwater, which clearly indicates that there is a change in its feeding habits. The larvae, fry and fingerlings enter tidal mud-flats, canals and estuaries. There is nothing on record to show when they go back to sea. The genus *Chanos* is thus an interesting one, which follows certain cyclic pattern in its life-history in different ecological conditions.

Like the majority of Clupeid fishes it has been customary to classify the Milk-fish as a plankton feeder. This is to some extent supported by the edentulous nature of the buccal cavity and the presence of long gill rakers which serve as filtering apparatus.

Studies on the gut contents of adult *Chanos* point out that it feeds on a variety of organisms, both plant and animal. It would be worthwhile to analyse these in detail here. Tampy (1958) critically examined the food items in different age groups of *Chanos* and came to the following conclusions. In the food of the fry and the fingerlings diatoms constitute the major portion and in the juveniles, there is a dominant occurrence of blue green algae but a relatively smaller proportion of diatoms. Nematode worms, spironid larvae (polychaetes), crustacean eggs and larvae, copepods, small gastropods and spats of Lamellibranchs constitute the chief food items in the adult fish. In brief, diatoms constitute only a relatively small proportion, compared to other algae in the adult gut (Tampy, 1958). It has also been observed that there is always an admixture of sand and mud particles. The above analysis points out that the food and feeding habits of *Chanos* differ in pattern from fry to adult. By the present study it has been possible to correlate the morphology and histology of the various parts of the alimentary tract to the feeding habits of the fish.

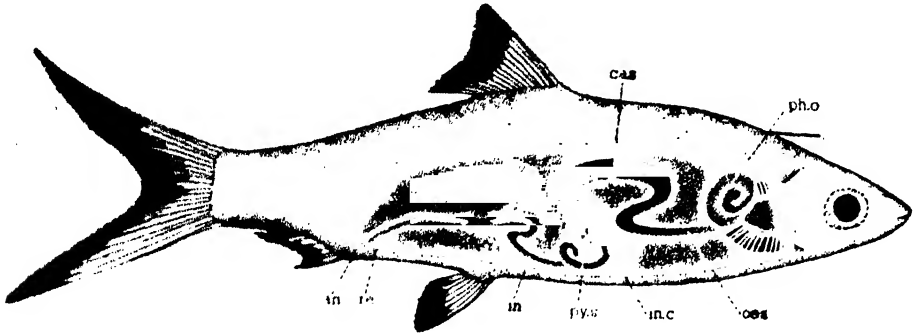
GROSS ANATOMY

The alimentary tract of *Chanos* consists of the buccal cavity, pharynx with pharyngeal organs, oesophagus, stomach, intestine with its numerous caeca and rectum (Fig. 1). The buccal cavity and pharynx constitute the organs of ingestion or the 'Kopfdarm' while the rest of the organs are the 'Rumfdarm' of Banki (1936) (op. cit. Brown 1957). The oesophagus and the stomach together form the foregut, the intestine, the midgut, and the rectum, the hindgut. Each of the major regions of the digestive system of this fish is distinctly demarcated externally and is therefore seen easily in gross anatomy.

The mouth is small, transverse and terminal. It is bounded by jaws which are without teeth. None of the bones of the cranium or branchial arches bears teeth. The buccal cavity is small and immediately leads to the more spacious pharynx. The gill arches are four in number and each one bears on its inner border rows of gill-rakers which are united at their ends by a common connective tissue. The gill-rakers are numerous, close-set, long and slender. Those of the successive arches intersect the gill slits so as to form an effective sieve.

A distinctive feature of the pharynx of *Chanos* is the presence of a pair of pharyngeal pockets, formerly known as 'gill-helix' or "suprabranchial organs"—a feature also shared by other Clupeid genera, like *Gadusia*, *Hilsa* and *Dorosoma*. The pharyngeal organ consists of a highly muscular sac and a canal passage, internally lined by rakers. Anatomical and histological studies point out that these organs are diverticula of the pharynx. (For a full description, see Kapoor, 1954a).

Microscopical study of the contents of the pharyngeal organ shows the presence of food particles along the canal passage as well as in the sac. Carmine-fed fingerlings corroborated these findings, pointing out that the organ is concerned in collection and direction of food from the pharynx to the oesophagus.



TEXT-FIG. 1.

Diagram showing alimentary canal of *Chanos chanos* (Forsk.). *an.*, anus *cas.*, cardiac stomach; *in.*, intestine; *in.c.*, intestinal caeca; *oes.*, oesophagus; *ph.o.*, pharyngeal organ; *re.*, rectum.

After giving off the pharyngeal organs, the alimentary tract narrows considerably and opens into the oesophagus. The oesophagus pierces the septum transversum and emerges into the peritoneal cavity. The oesophagus is a straight, narrow, somewhat flattened tube, of more or less uniform calibre, with a fairly thick wall. On opening the oesophagus by a median longitudinal incision, it is seen that the mucosa is raised into a series of closely arranged folds, about 20-22 in number, making a spiral coil, like the threads of a screw along the whole length of the oesophagus. (For full details, see Chandy, 1956).

Following the connotation of the comparative anatomists, the stomach or ventriculus in a vertebrate animal is defined as that part between the terminus of the oesophagus and the beginning of the intestine. Accordingly, the stomach in *Chanos* occupies an extensive portion of the alimentary canal, between the oesophagus anteriorly and the cluster of intestinal caeca, posteriorly. On uncoiling the alimentary canal, the stomach is seen to consist of two distinct parts, the corpus or proventriculus and the pylorus or gizzard. The corpus is the descending loop and the pylorus the ascending loop, which run parallel to the oesophagus and at the anterior level of the latter, continues into the intestine.

The corpus portion of the stomach presents differential thickening, which is discernible externally and the presence of three distinct zones in it has been corroborated by histological study. The pyloric division, demarcated by the pyloric constriction, is modified into a pyriform gizzard with glistening wall. The gizzard is a highly muscular organ with a narrow lumen which communicates with the intestine.

The intestine is a long narrow tube which falls into many convolutions. It does not show differentiation into duodenum and ileum. The anterior region, following the gizzard, receives internally a series of openings about 18 in all, which are arranged in three piles. These openings communicated with the intestinal caeca, numbering about 120 to 150. Intestinal caeca are simple or branched finger-shaped organs of different lengths. They form a compact cluster and lie in the space between the pyloric stomach and the loops of the intestine. It is more or less of uniform diameter and passes imperceptibly into rectum, which is not morphologically different from the intestine.

The liver is a bilobed organ, extending over the entire length of the oesophagus which it conceals. The gall bladder is situated in between the two liver lobes. The bile duct takes a straight course posteriorly and opens into the first part of the intestine amidst the cluster of intestinal caeca. In vertebrates, the location of the opening of the bile duct lends a clue to the exact boundary between the stomach proper and intestine.

The pancreas is diffused and the different patches are scattered in the mesenterial folds surrounding the intestine and the intestinal caeca, which are also interspersed by adipose tissue. The lobes of pancreas are seen in transverse sections adhering to the intestine.

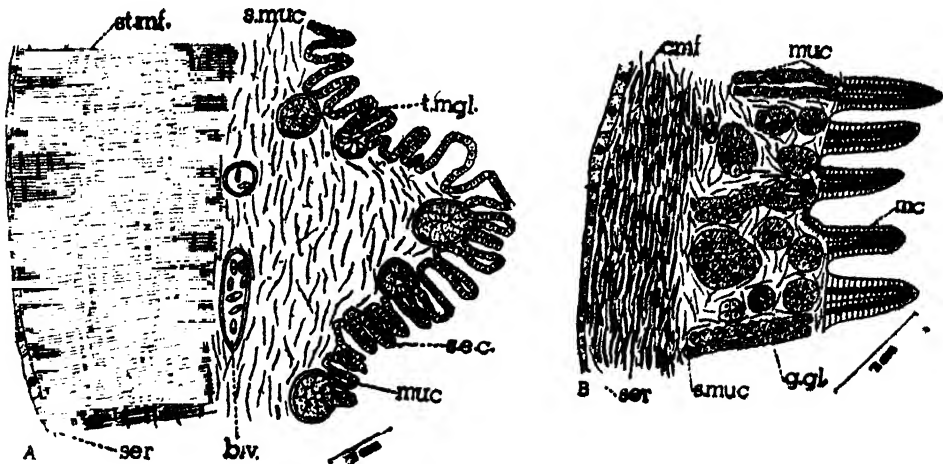
HISTOLOGY

The histology of the buccal cavity, pharynx and pharyngeal organ has already been dealt by Kapoor (1954a) and that of the oesophagus by Chandy (1956). The present account, therefore, deals with the histology of the rest of the alimentary tract, posterior to oesophagus.

The Stomach :

The wall of the stomach is constituted by the usual four layers, viz., serosa, muscularis, submucosa and mucosa. These layers are by no means uniform throughout the extensive stomach. Their variations bring about some arbitrary differentiations, that one notices three distinct zones in the corpus as already described. The first is the anterior, transitional zone immediately following the oesophagus, the second or the middle zone lies in the descending loop and lastly the third zone which adjoins the gizzard.

The serosa is a narrow but distinct layer of membraneous tissue, with a large number of nuclei, arranged in two to three piles. Blood vessels course between serosa and the layer of muscles beneath. The muscular layer is differentiated into an outer longitudinal and an inner but broader circular bands. Submucosa is a loose network of connective tissue and is highly variable in the different zones. Mucosa, the most characteristic tissue of the stomach, is made of columnar epithelial cells which have undergone modifications to form mucous and gastric glands.



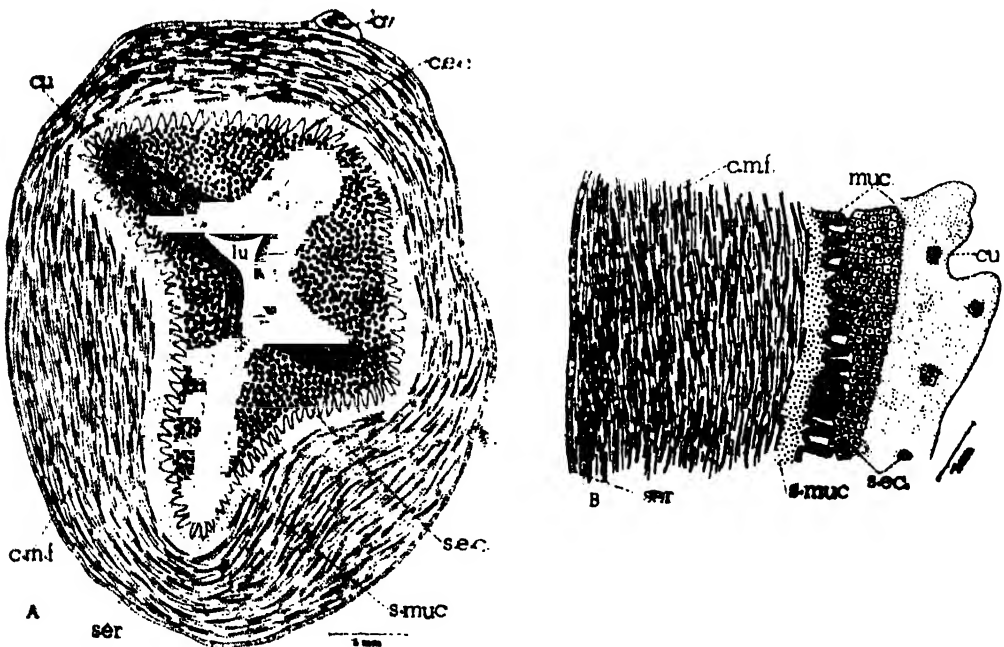
TEXT-FIG. 2.

Transverse sections—A. Transitional zone. B. Cardiac stomach of *Chanos*. b.v., blood vessel; c.m.f., circular muscle fibres; g.gl., gastric gland; muc., mucosa; m.c., mucous cell; s.e.c., stratified epithelial cells; st.m.f., striated muscle fibres, s.muc., submucosa; t.m.gl., tubular mucous gland.

While the general histology of the stomach is as described above, slight variations in the three zones are noteworthy. In the transitional zone which is between oesophagus and stomach proper, the musculature is thick and the muscle fibres are distinctly of the striated nature. The submucosa is a broad layer with numerous large capillary vessels. In the submucosa there are present tubular glands with giant cells. Each gland is made up of cluster of cells, which are filled with a clear, hyaline fluid. The nucleus is basal and it takes a light stain with haematoxylin. They differ in size and their staining reaction from the mucous cells of the oesophagus and the gastric glands to be described later. Whether these giant glands of the first zone produce only mucous—they do give a positive test with mucicarmin—or whether they produce any enzyme, it is difficult to comment from mere histology. The mucosal epithelium resolves into numerous villi-like structures. The cells at the distal free end of the villi show complete stratification (Fig. 2A).

The middle zone presents a very characteristic and interesting feature. The columnar epithelium is modified to form the gastric glands which sink deep below the inner border. The glands are tubular and branched. The glands are numerous and form a very compact mass, occupying a major belt in the thickness of the wall (Fig. 2B). A comparatively thick zone of muscles, resolved into an outer longitudinal and an inner circular layers, surrounded the glandular belt. The muscles are of the striated nature as in the first region of the stomach. But as the corpus passes into its last zone, a change from striated to smooth muscles is clearly visible. This is an interesting feature of the histology of the stomach and points out that a major portion of the corpus is under voluntary control of the fish.

The third or the last zone is characterised by an extremely thin peritoneum and a thin smooth muscle layer, where circular layer of muscles is distinctly seen,



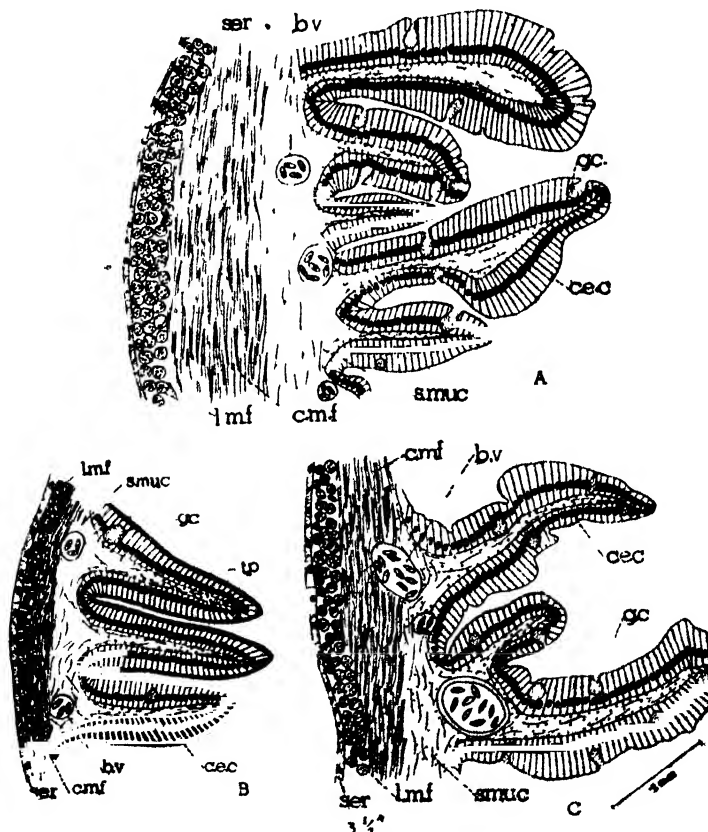
TEXT-FIG. 3.

Transverse sections—A. Pyloric stomach of *Chanos*. B. Same much enlarged *b.v.*, blood vessel; *c.e.*, columnar epithelial cell; *cu.*, cuticle; *c.m.f.*, circular muscle fibres; *lu.*, lumen; *muc.*, mucosa; *ser.*, derosa; *s.e.c.*, stratified epithelial cells; *s.muc.*, submucosa.

while the longitudinal muscle fibres are seen with difficulty. The mucosa is an insignificantly thin layer with little or no glands.

The wall of the pylorus or gizzard is composed of the same series of coats as those of the corpus, viz., serosa, muscularis, submucosa, and mucosa (Fig. 3A). But each tunic in it has undergone great modifications. The serosa is very much reduced and consists of few layers of cells. Blood capillaries are seen to course along it. The muscular layer reaches the maximum degree of specialisation. This layer is composed solely of circular bundles of smooth muscle, the longitudinal muscle bundles being totally absent. The hypertrophied circular layer of muscles thus constitutes the bulk of the wall.

The submucosa is quite distinct and subtends the villi of the mucosa. The mucosa is a characteristic feature of this region. It is disposed into a large number of villi-like folds which are composed of epithelial cells with prominent nuclei. No glands are present. Inner to the mucosal layer are discernible a stratified epithelial layer and a layer of non-cellular cuticle or keratinised tissue. Scattered in the matrix of the cuticle are groups of isolated cells broken off from the epithelium below. The cuticle shows a tendency to become more and more dense towards the lumen (Fig. 3B). It may be mentioned here that in finger-lings of 3½–4" length the cuticular layer is absent in the gizzard.



TEXT-FIG. 4.

Transverse sections—A. Intestine. B. Intestinal caeca C. Rectum of *Chanos b.v.*, blood vessel; c.m.f., circular muscle fibres; c.e.c., columnar epithelial cells; g.c., goblet cell; l.m.f., longitudinal muscle fibres; muc., mucosa; s.muc., submucosa; t.p., top plate.

The Intestine :

The spacious lumen of the intestine is surrounded by the four layers, serosa, muscularis, submucosa and mucosa (Fig. 4A).

The serosa is a delicate membrane enveloping the muscularis. The muscularis is composed of an outer longitudinal and an inner circular layer, both of non-striated nature. The submucosa is a thin layer ramifying into the mucosa. Blood corpuscles, evidently of the leucocytes series, are scattered in the submucosa. The mucosa is a prominent layer of columnar epithelium and is characterised by simple or branched long villi. The epithelial cells are narrow and long with basal nuclei and are arranged compactly. Large mucous cells, in various stages of activity, is a regular feature throughout the coils of the intestine. No glands have been observed. Blood capillaries laden with corpuscles are abundant.

The Intestinal caeca :

Microscopical study of the intestinal caeca points out to the fact that they are extensions of the intestine. The histology of both the organs is very much alike. However, a noteworthy difference is the comparatively thinner musculature in the caeca. The mucosal villi are unbranched, more slender and pointed. Free border has been detected in various preparations. Mucous glands are present in large numbers in the caeca. (Fig. 4B).

The Rectum :

That the last portion of the intestine is modified into a distinct rectum is amply proved by its histology. The usual four layers are present.

The serosal layer is thin but distinct. The muscularis is much thicker than that of the intestine and the circular layer of muscles is more conspicuous than the longitudinal layer. The submucosa is a loose network made of an anastomosing strands of connective tissue, with a large number of vacuolated spaces and rich in vascular supply. The mucosa made up of columnar cells, falls into broad, blunt villi, simple or branched. Large unicellular goblet cells are present all along the mucosa (Fig. 4C).

DISCUSSION

In reviewing the knowledge of the digestive system in relation to food and feeding habits of *Chanos*, it is necessary to compare it with other members of its order. Like the majority of the Clupeids, the mouth is edentulous. The gill rakers form a sieve to strain the food particles from the incurrent water. Like *Hilsa*, *Gadusia* and *Dorosoma*, *Chanos* also possesses pharyngeal pockets which according to the authors are food procuring device.

The presence of a spiral mucosal fold in the oesophagus, with its rich supply of mucous glands is a unique feature in *Chanos*. The transition zone, between the oesophagus and the cardiac stomach, has in its submucosa many giant tubular mucous glands. The copious production of mucus may be correlated to the composite food items of diatoms, other algae, gastropods, lamellibranchs and concomitant admixture of sand and mud.

The stomach is concerned with the storage of food and gastric digestion. The size for storage is influenced by two factors : the nature of the food, and the duration of intervals between the meals. Tamy (1958), reports that he noticed in the stomach of some of the adult fishes, only mucoid matter in the form of a white paste, and came to the conclusion that the fishes were starving. From this it may be surmised that *Chanos* may be an irregular feeder. The stomach of *Chanos* is considerably large and complex in comparison to the rest of the alimentary canal.

The size of the stomach and the capacity of the cardiac zone for dilatation point out that it serves a very important function of food storage. The abrupt changes in the direction of loops of the stomach obviously check the quick passage of food from the stomach to the intestine.

The extension of the striated muscles of the oesophagus over part of the stomach has been observed in a few fishes. The presence of such muscle fibres in *Chanos* gives the anterior region of the stomach voluntary control, whereby the food could be regurgitated whenever necessary.

Histological studies reveal that there is a large area occupied by the gastric glands. This enables the fish to secrete large amounts of gastric juice making the gastric digestion efficient, before the food is passed on to the gizzard.

A highly muscular gizzard (grinding mill) is present in *Chanos*. The most striking feature in the histology of the gizzard is the presence of two supplementary layers, the stratified and the cuticular layers, apparently developed from the epithelium itself. That the cuticle arises by the modification of the epithelium is amply supported by the presence of detached and scattered cells in the cuticle. In the gizzard, following the mucosal epithelium, there is a region of stratified epithelium, an adaptation to withstand its wear and tear. It may be mentioned here that since the stratified epithelium is inefficient as an absorptive membrane, the gizzard only performs the function of trituration. A cuticular region is present and it affords protection from the gritty and hard food materials the fish takes. The first layers of the cuticle, by fusion of contiguous areas, form the innermost dense cuticle of the gizzard. All the previous authors, who have worked on the gizzard of fishes, are of opinion that the cuticular layer is formed by the secretion of mucous glands. But in *Chanos* such glands are totally absent in the gizzard. The present study points to a different mode of cuticle formation in gizzard. The cuticle in *Chanos* gizzard arises by stratification and cornification of the epithelium, as is the method of origin of cuticle in the epidermis of skin.

Differences in the nature of the food of young and adult *Chanos* have been observed by Tamy (1958). He points out that the adult fish takes more of coarser food particles, whereas the young of *Chanos* is exclusively a plankton feeder. The histology of the gizzard of the fingerlings shows that all the layers of the adult fish, except the cuticular layer, are present. The acquisition of the cuticle in the gizzard by the adult fish is a subsequent adaptation when the change-over takes place to a harder type of food.

Blood corpuscles in large numbers scattered in the submucosa are present in the intestine. It may be inferred that as in other Teleosts, the wall of the intestine is a haemopoietic organ.

The free border which is an additional layer of absorption is absent in the intestine and rectum. But its presence in the intestinal caeca supports the view that it is primarily an absorption region.

Several fishes, belonging to the different orders, are known to possess gizzard. One of the well known orders with gizzard is the Mugilidae. Gazzawi (1935), Ishida (1935), Pillay (1953), and Mahadevan (1950) have worked out in detail the digestive system of a few species of *Mugil*. Among the Clupeids the gizzard of *Dorosoma cepedianum* and *Gadusia chapra* have been described by Weir and Churchill (1945), and Kapoor (1958) respectively. The authors (unpublished) have observed the presence of a gizzard in *Colisia fasciata* of the order Anabantidae. All these genera, though unrelated phylogenetically, show similar modifications. The similarity is explained on the basis of their feeding habits. *Mugil* sp., *Gadusia chapra* and *Colisia fasciata* are mudfeeding forms, grovelling in mud for organic debris, algae and plant tissues. *Chanos* also takes in a good amount of mud and sand along with diatoms, other algae and molluscs. This appears an interesting case of convergence in evolution.

Chanos is thus a mixed feeder, a habit, probably it has acquired because of its migratory habits. Its digestive system recalls that of a grain-eating bird, with its crop, large cardiac portion and well developed gizzard. The structure of the stomach is elaborate in *Chanos*. The zonal differentiation is marked and the gastric glands are highly developed. The giant mucous cells, the massive complicated gastric glands, and the powerful gizzard with cuticular lining are indications of an advanced state of physiological activity.

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RIBONUCLEIC ACID IN THE OOCYTES OF THE ASCIDIAN *PYURA* SP. (PYURIDAE : PLEUROGONIA)

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ABSTRACT

The 'test cells' are characteristic of the ascidian ovum. In *Pyura* sp., they do not occur in the early oocyte but appear much later in its peripheral cytoplasm. They are rich in ribonucleic acid. In later stages of oogenesis, they break up and release their RNA content into the cytoplasm of the developing oocyte. In addition to the nucleolus and the oocyte cytoplasm, which are important sources of RNA, the test cells form a third source. Cytochemical reactions indicate that the test cell RNA is different from that of the nucleolus and oocyte cytoplasm.

INTRODUCTION

One of the basic facts of modern cytology and cell physiology is the establishment of an undeniable association between ribose nucleic acid (RNA) and protein synthesis in the animal cell. Independently put forward by Caspersson (1941) and Brachet (1942) this view has gained increasing support in view of the evidence that has accumulated in recent years. Recently, Brachet (1957) has reviewed the problem and has summarized the experimental evidence in favour of it. The origin of the RNA in the cell, however, has been the subject of debate and at least two sources are cited, (a) the microsomes in the cytoplasm and (b) the nucleolus in the nucleus. Both are rich in RNA and evidence that either or both are sources of its origin has accumulated during recent years (Brachet, 1957). One of the most striking is that presented by Fauré-Fremiet *et al.* (1950) who, in their studies of the developing oocytes of *Glomeris* have clearly established this dual origin of RNA. It is more or less clearly understood that whenever synthesis and growth are taking place in the cell, abundant amounts of RNA are present in the cytoplasm and in the nucleus (in the nucleolus). Other possible sources of RNA have been occasionally investigated but have not been established. In this context, the ascidian egg offers special problems. The "inner follicle cells" or "test cells" of the ovum are peculiar to the ascidian and their role has not been established. Berrill (1950) states, "The inner follicle cells, often called by the ambiguous term "test cells", have long been the subject of controversy with regard to the role they play in the growth of the ovum" (page 36).

Recent examination of oogenesis in an Indian ascidian *Pyura* sp. (Pyuridae Pleurogonia) has offered some ideas which are presented in this paper.

MATERIAL AND METHODS

The material used in the present study was collected in January 1958 at Madras. The gonads were fixed in Carnoy's and Sanfelice's fluids. They were cut at varying thickness and stained with the Feulgen reagent and counterstained with light green. Methyl green-pyronin and toluidine blue were the other stains employed. Digestion with trichloroacetic acid and ribonuclease was also done in order to establish the areas of presence and distribution of RNA.

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OBSERVATIONS

The Oocyte : Examination of young oocytes (up to $60\text{--}75\mu$ diameter) shows that their cytoplasm is rich in RNA as evidenced by its strong affinity for both toluidine blue and pyronin (Pl. VIII, fig. 1). One of the striking features of this early development is the gradual increase in size of the nucleolus which reaches a maximum diameter of 10μ when the cell is 60μ , after which no further increase appears to take place (Rao, 1959). In later stages of growth, when the oocyte attains a diameter of 75μ or more, there is a gradual decrease in cytoplasmic basophilia. When the oocyte reached a diameter of $90\text{--}120\mu$ there appears a peripheral zone in the cytoplasm exhibiting strong basophilia (Pl. VIII, fig. 3). Extraction with ribonuclease and hot trichloroacetic acid confirms that this zone is rich in RNA. This peripheral zone is also rich in mitochondria.

The Test Cells : These are characteristic of the ascidian oocyte and occur in its periphery. They are rounded cells with small nuclei (Text fig. 1 and Pl. VIII, fig. 2). They seem to project into the cytoplasm of the oocyte but are distinct from it and they possess a clear cell membrane. During early stages of the development of the oocyte, i.e. when its diameter is less than 60μ , the test cells are few and inconspicuous. Later, they grow larger and form characteristic structures of the oocyte. Their cytoplasm gradually accumulates basophilic material in the form of large granules which fill the entire cell. The basophilia is so intense that only in Feulgen preparations or in those subjected to extraction by ribonuclease or hot trichloroacetic acid can the nucleus be seen (Pl. VIII, fig. 2). The nucleus is small and has a number of coarse chromatin granules. Sometimes the test cells migrate into the cytoplasm of the oocyte, and occasionally several test cells are seen close to the nucleus (Text fig. 1).

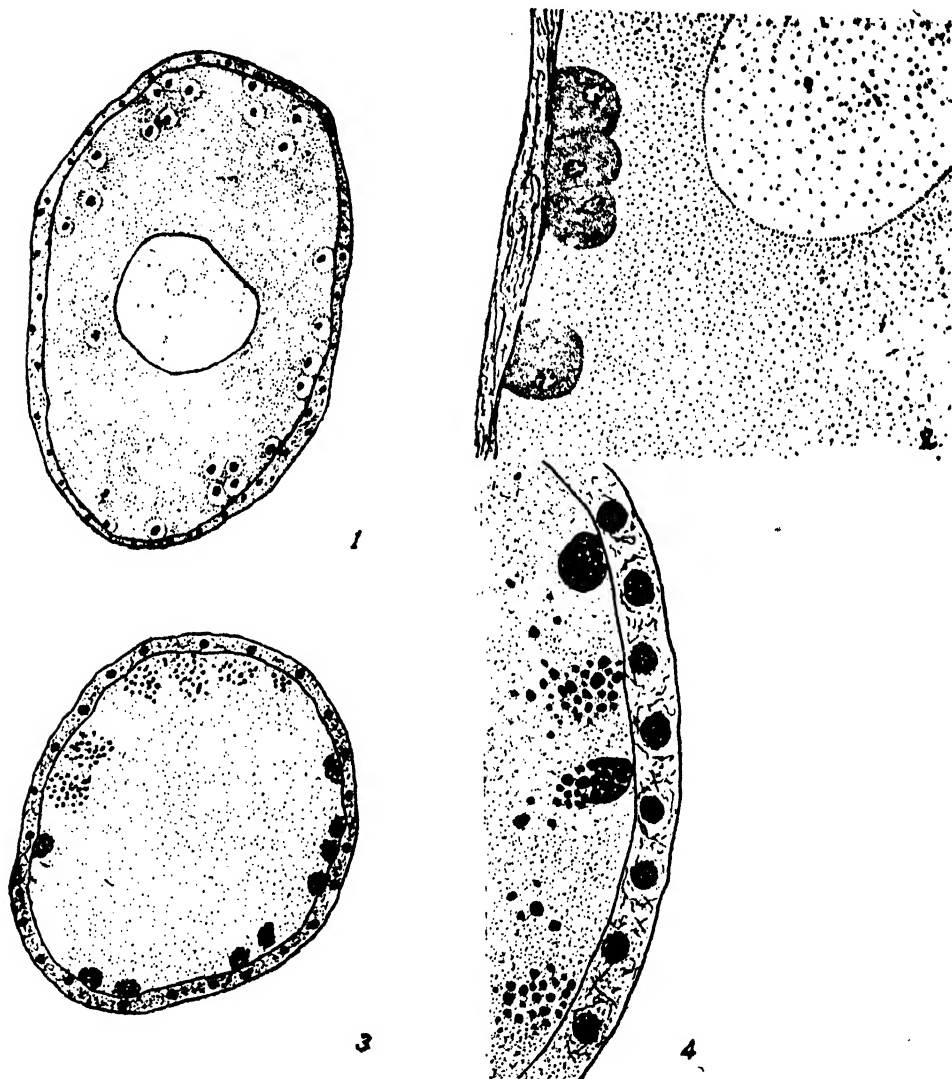
Treatment with ribonuclease and hot trichloroacetic acid (see Seshachar, 1950, for method) shows that the basophilia of the oocyte cytoplasm as well as that of the nucleolus becomes considerably diminished, and on prolonged treatment, the basophilia is lost altogether. On the other hand, the basophilia of the cytoplasm of test cell is not extracted easily. In preparations stained with toluidine blue after extraction with 5 per cent trichloroacetic acid at 80°C for 15 minutes, the oocyte cytoplasm and nucleolus are perfectly clear, but the test cells are still dark though they are not so dark as to obscure the nucleus (Text fig. 2). Extraction with ribonuclease is even slower. After one hour's digestion, the cytoplasm and nucleolus are clear but the test cells exhibit basophilia to a relatively high degree. Only prolonged extraction with trichloroacetic acid at 90°C removes basophilia altogether from the test cells.

The later fate of the test cells is interesting. In oocytes of diameter of $90\mu\text{--}120\mu$, the test cells are seen to break up and release their basophilic granules into the peripheral cytoplasm of the egg (Text figs. 3 & 4). The intense basophilia noticed in the egg periphery at this stage is almost entirely due to this. Stages in this disintegration are quite easily seen. The nucleus also disintegrates later. Older oocytes do not show the test cells in their cytoplasm.

DISCUSSION

In the developing egg of *Pyura*, there are at least three sources of RNA. In early stages, the intense basophilia of the nucleolus and cytoplasm indicates the presence of ribonucleotides in these two cell constituents. It was pointed out earlier (Rao, 1959) that the growth of the nucleolus reaches a maximum when the oocyte diameter is about 60μ , and thereafter remains stationary. At a slightly later stage when the oocyte diameter is about 57μ , the cytoplasmic basophilia starts declining. Again, when the oocyte reaches a diameter of $90\mu\text{--}120\mu$, there appears a ring of intensely basophilic granules in its periphery. Apparently in the early

stages of growth, the source of RNA is the nucleolus and the general cytoplasm. Later stages are characterised by a third source, i.e. the test cells, which have all along been accumulating RNA in their cytoplasm and which now break up releasing their contents into the cytoplasm of the oocyte.



TEXT-FIG. 1.

- Fig. 1. Oocyte of *Pyura* sp. showing 'test cells' in the cytoplasm Feulgen. $\times 1260$.
 Fig. 2. Part of oocyte of *Pyura* treated with 5% Trichloroacetic acid at 80°C for 15 minutes and stained with toluidine blue. The oocyte cytoplasm is clear but the test cells show basophilia. $\times 2800$.
 Fig. 3. Oocyte at a later stage with the test cells breaking up and releasing their basophilic granules into the cytoplasm. Toluidine blue staining after ribonuclease treatment. $\times 840$.
 Fig. 4. Part of the same showing the large, coarse granules in the peripheral cytoplasm of the oocyte. Toluidine blue staining after ribonuclease treatment. $\times 1850$.

There is reason to believe that the RNA of the test cells is in some way different from that of the oocyte nucleolus and cytoplasm. Its reactions to trichloroacetic acid and ribonuclease would indicate this. Vincent (1957) has recently provided evidence of a heterogeneity of RNA in the starfish oocyte, and it is possible that in *Pyura* also, the RNA is of more than one kind.

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EXPLANATION OF FIGURES IN PLATE VIII

- Fig. 1. Section of ovary of *Pyura* sp. stained with toluidine blue. The more pronounced basophilia of the younger oocytes is clearly seen. $\times 100$.
 Fig. 2. Oocyte showing test cells in its periphery. The young oocyte in the lower left corner has none. Ribonuclease treated. Toluidine blue. $\times 350$.
 Fig. 3. Part of oocyte at a later stage showing peripheral accumulation of intensely basophilic granules.



SOME ASPECTS OF BRACHIOPOD RIBBING

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ABSTRACT

Variation in numbers of costae on brachial valve and on pedicle valve of *Goniorhynchia Boueti goniaeae*, has been studied by means of various statistical methods. In the population of the species studied, on an average, pedicle valve possesses one more costa than brachial valve, although reverse as well as same numbers of costae on both valves have been found to be true for a few specimens. Accordingly the population has been grouped into three divisions having the normal, the reversed and the neutral ribbing system.

Relation between numbers of costae on the brachial fold and the plane of symmetry of shells has also been studied. There may have been both symmetrical as well as asymmetrical forms according to the presence of odd or even numbers of costae on the brachial fold. Considering the position of the plane of symmetry, on costae or on sulci, occurrence of two types of symmetrical, the normally symmetrical and the reversed symmetrical forms has been suggested. No correlation has been found between the ribbing system and symmetry elements.

INTRODUCTION

The external surfaces of many brachiopod shells consist of radiating ribs from the umbo. Such ribs are designated as *Costae* and the depressions that separate two costae as *Sulci*. The brachiopod genera belonging to the superfamily Rhynchonellacea possess as a rule such radiating costae, although the presence of such character in other superfamilies is not rare. The ribbing system in brachiopod is quite important, as the rib characteristics, particularly the number of ribs on the brachial fold, have often been used in the taxonomy of the genera belonging to the Rhynchonellacea superfamily. In the present work an attempt has been made to study the relation between number of costae on one valve to that on the other in *Goniorhynchia boueti* Davidson. A collection of individuals of the species shows variation in the character studied. Hence the use of statistical methods becomes inevitable. Finally some suggestions have been made regarding the position of the plane of symmetry in relation to the brachial fold.

HISTORY AND MATERIAL

During recent years brachiopods have been subjected to various statistical investigations. Most of the workers have concentrated on general metrical characters (such as length, width, thickness etc). Very little work has so far been done to investigate the variation in the numbers of costae in brachiopods.

Cumings and Mauck (1905) have studied the numbers of costae in *Platystrophia lynx* Martin, from the Upper Ordovician of Vevay, Indiana. They have studied the numbers of Costae on both valves and separately on the fold as well. In their collections it has been found that the brachial valve possesses one more costa than the pedicle valve.*

*From the available data it has not been possible to find whether this holds good for every individual or not. Their conclusion was drawn from the arithmetic means of the two distributions. It is difficult to comment upon the statistical significance of the difference, as parameters available have been found to be inadequate to carry out such comparison.

Wilson (1914) employed the variation in the numbers of plications (can be taken as equivalent of costae) in *Parastrophia hemiplicata* Hall for stratigraphic sub-division.

Bancroft (1928, 1945) made an elaborate study of ribbing in a group of brachiopods belonging to the superfamily Dalmanellacea. He has evolved a special ribbing notation to study the nature of the splitting of costae. It is worth mentioning here that such variation has successfully been employed to subdivide the Lower Ordovician of Great Britain.

Parkinson (1954) made a comparative study of the numbers of costae on the brachial fold of *Pugnax* in three different collections from the Lower Carboniferous of Great Britain.

It can be seen from the above survey, with the exception of Cumings & Mauck (loc. cit.), that none of the investigators attempted a comparative study of the numbers of costae as between two valves, viz. the brachial and the pedicle valves of individuals from the same collection.

The present work is an extension of the investigation carried out by Mitra (1956, 1957). The relevant portion from that work is summarised below for convenience.

Three collections of *Goniorhynchia boueti* Davidson — Langton Herring, Burton Bradstacks and Eype Mouth were made from Dorset in South England and the fourth one from Normandy in France. Langton Herring and Eype Mouth collections were made by the author and the rest borrowed from the British Museum (Natural History). In all the four collections, the numerical value of the arithmetic mean of the distribution of the number of costae on the pedicle valve is one greater than that of the costae on the brachial valve. The comparison of means by the usual method of pooling the standard errors of means is not applicable here, as the variates show a high degree of correlation. A different method (see below) has been used in the present work.

As the purpose of the present work is to study the interrelation between the numbers of costae in two valves, rather than a comparative study as between collections, a single collection out of the four has been used. The Eype Mouth collection has been found suitable for this purpose. The original collection from Eype Mouth consists of 100 specimens. Mitra (loc. cit.) used only 60 specimens for the brachial valve and 66 specimens for the pedicle valve. Counting of costae on other specimens was not possible because of imperfect preservation. The number of individuals in the sample, used in the work under study, was further reduced as the counting of costae on both valves of the same individual, was possible with 46 specimens only. No marked difference in the estimated parameters was observed between this sample and the original collection of Mitra.

ANALYSIS

The numbers of costae on the brachial and the pedicle valves have been plotted to get their frequency histograms (Fig.1). The statistics of arithmetic mean (\bar{x}), standard error of the mean (s/\sqrt{n}), standard deviation (s) and coefficient of variation ($100 s/\bar{x}$) have been estimated in the conventional way and are given in Table I, while original data are given in Table II. In all cases unit group intervals have been taken.

In both the brachial and the pedicle valves the frequency distributions are unimodal, and from the estimation of the coefficient of variation it is apparent that the numbers of costae in both valves are highly variable. The arithmetic mean of the costae distribution of the brachial valve is approximately one less (0.87) than that of the pedicle valve. To make sure that the observed difference between the means is inherent in the collections and not due to chance in random sampling, the following methods have been used as checks.

TABLE I

Distribution of number of Costae on the brachial and the pedicle valves

	Brachial Valve	Pedicle Valve
Mean	30.43 \pm 0.50	31.30 \pm 0.51
Standard Deviation	3.42	3.51
Coefficient of Variation	11.22	11.23
Number in sample	46	46

TABLE II

Number of Costae (Original Data) X_b —Costae on Brachial valve X_p —Costae on Pedicle valve.

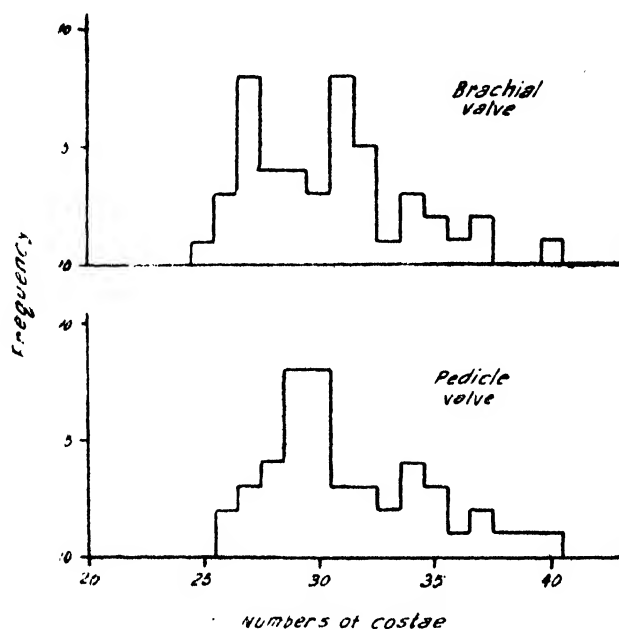
No. Sp.	X_b	X_p	$X_p - X_b$	No. Sp.	X_b	X_p	$X_p - X_b$
1	27	26	-1	24	28	29	1
2	32	34	2	25	31	30	-1
3	30	31	1	26	25	29	4
4	31	31	0	27	36	39	3
5	27	29	2	28	28	27	-1
6	31	30	-1	29	31	35	4
7	28	29	1	30	27	28	1
8	33	34	1	31	35	35	0
9	30	30	0	32	27	27	0
10	31	32	1	33	31	33	2
11	34	35	1	34	34	33	-1
12	35	36	1	35	27	29	2
13	32	32	0	36	27	26	-1
14	34	37	3	37	31	30	-1
15	40	40	0	38	31	30	-1
16	26	28	2	39	29	30	1
17	27	28	1	40	32	34	2
18	37	37	0	41	37	38	1
19	29	30	1	42	26	28	2
20	32	34	2	43	29	32	3
21	29	29	0	44	26	27	1
22	32	31	-1	45	28	29	1
23	30	30	0	46	27	29	2

TABLE III

Frequency distribution of $(x_p - x_b)$

$x_p - x_b$	-1	0	1	2	3	4
Frequency	9	9	14	9	3	2

The number of costae on the pedicle valve (x_p) in each of the 46 specimens has been subtracted from the number of costae on the corresponding brachial valve (x_b). The mean, standard error and other parameters of the $(x_p - x_b)$ -distribution have been estimated. Any significant deviation of the mean of this distribution from any assumed value can be tested by t -test with the help of its standard error. Results have been given in Table IV.



TEXT-FIG. 1.

The mean of this distribution (0.87) is the same as before; it is positive and the deviation from zero is statistically significant ($p < 0.01$).

TABLE IV
Distribution of $(x_p - x_b)$

Mean	0.87 ± 0.20
Standard Deviation	1.34
Coefficient of Variation	154.68
Number in the Sample	46

As a second step of analysis, correlation between two variates have been estimated with the help of sum of products. In the Table V it can be seen that the correlation coefficient is very high (0.92). Finally by considering x_b as x and x_p as y a linear reduced major axis has been fitted. Reduced major axis has an advantage over the conventional regression line. (Kermack and Haldane 1950, Imbrie 1956). The satter-gram is shown in figure 2.

It is clear from the reduced major axis coefficient (1.03) that an expected value of x_p is approximately one greater than a given value of x_b , provided the given value is not too small. The lowest observed limit of x_b is 25, while the highest limit is 40. Within this observed range any given value of x_b yields an expected value of x_p approximately one greater than x_b . It is not likely that lowest limit of x_b , in any other samples derived from the same population will have much lesser value.

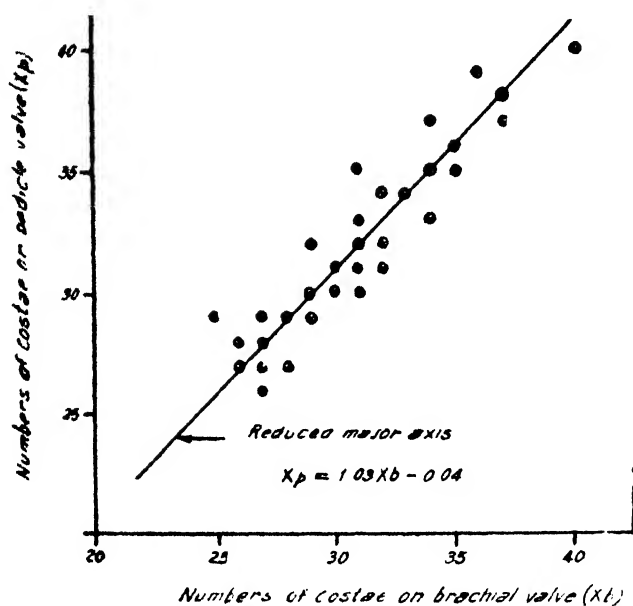
TABLE V

Correlation between numbers of Costae on brachial and pedicle valve

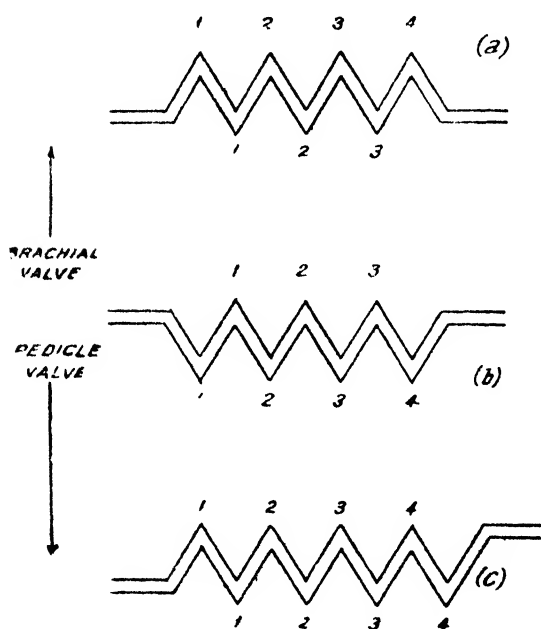
Numbers of																			
Costae	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	Total		
Pedicle Valve	40															1	1		
	39											1					1		
	38												1				1		
	37									1							2		
	36										1						1		
	35						1			1	1						3		
	34							3	1								4		
	33						1			1							2		
	32				1		1	1									3		
	31					1	1	1									3		
	30					2	2	4									8		
	29	1		3	3	1											8		
	28		2	2													4		
	27		1	1	1												3		
	26			2													2		
Total	1	3	8	4	4	3	8	5	1	3	2	1	2	0	0	1	46		
Brachial Valve																			
Correlation Coefficient									 0.92								
Reduced Major Axis									 $x_p = 1.03 x_b - 0.04$								

Considering both the comparison of means as well as the reduced major axis, coefficient it can be concluded that on an average the pedicle value has one more costa than the brachial valve.

The ribbing system of the brachiopods, taking both the valves together, is a bicorrugated system, that is, the sulci of one valve correspond with the costae of another. In such a system it is possible to demonstrate three types of structures (Fig.3). Figure 2b shows a ribbing system, wherein it is obvious that the pedicle valve should have one more costa than the brachial valve. Figure 2a shows the reverse phenomenon, that is, the brachial valve has one more costa than the pedicle valve. On the other hand in the ribbing system, as shown in Figure 2c, it is possible for both valves to have the same number of costae. Conveniently, the three systems



TEXT-FIG. 2.



TEXT-FIG. 3.

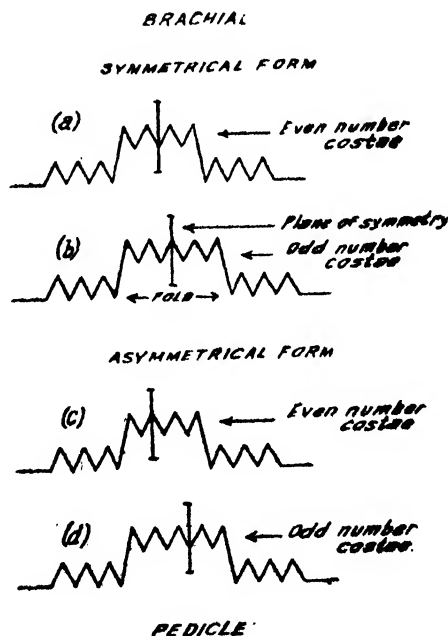
may be designated as the *Normal*, (Fig. 3b), the *Reversed* (Fig. 3a) and the *Neutral* (Fig. 3c) system respectively. In the present instance the pedicle valve having one more costa (Fig. 3a), has been taken as normal as the mean value of the pedicle distribution is approximately one greater than that of the brachial distribution. In other cases, for instance, in *Platystrophia lynx*, the second type is the normal type.

It may be mentioned that the ribbing system may be different in different genera, even in different species of the same genus.

The frequency distribution of the three types of system are given in Table III. More specimens have one more costa on the pedicle valve than on the brachial valve, in comparison with other varieties, taking independently. A few specimens have two, three and four more costae on the pedicle valve. Mercier (1937) and Mitra (loc.cit.) have noticed in a few specimens that the costae on either side of the main flanks near the pedicle sulcus often flatten out when they reach the anterior commissure. The *semi-costae* (Mercier loc. cit.) have no brachial counterparts. The anomaly of having two, three and four more costae on the pedicle valve may well be due to the presence of semi-costae.

NUMBER OF COSTAE ON THE BRACHIAL FOLD AND THE PLANE OF SYMMETRY

About 50 per cent specimens have odd numbers of costae on the brachial fold while the other 50 per cent have an even number. The brachiopods are, in general, bilaterally symmetrical animals. If therefore an individual possesses an even number of costae on the fold, the plane of symmetry of the shell is expected to pass along the groove between the two costae (Fig. 4a). On the other hand in an individual with an odd number of costae one would expect the plane of symmetry to pass along the central costa, having thereby a reversed form (Fig. 4b) with respect to the plane of symmetry. As about 50 per cent of individuals have one and the other 50 percent the other of the two types of form, the *normally symmetrical* and *reversed symmetrical* forms, are randomly distributed in the population.



TEXT-FIG. 4.

The above three groups with distinctive ribbing systems, have also been studied separately for odd and even numbers of costae on the fold. A similar proportion

of odd and even numbers of costae have again been found within each of the three groups. Occurrence of both odd and even numbers of costae on the folds in each of the three groups in 50 percent proportion, shows lack of correlation between the ribbing system and the symmetry element. Specimens having the same ribbing system may have a different symmetry element. On the other hand, specimens having same symmetry element, may have different ribbing system.

An alternative (and probably more likely) explanation of the occurrence of both odd and even numbers of costae on the fold is that the fold itself is asymmetrical in the case of 50 per cent of specimens. Such asymmetry would result if the plane of symmetry invariably passes through a costa or between two costae (Fig. 4c and 4d). An attempt has been made to determine from transverse sections of shells whether the plane of symmetry (as defined by the median septum) lies along a costa or between adjoining costae. But the thickness of the shell makes comparison of the inside and outside inconclusive.

It may be mentioned here that such discrepancy in the plane of symmetry may also be caused by the total numbers of costae (whether odd or even). But the degree of asymmetry in that case may be considered negligible as the asymmetry of the shell is caused by a single costa, which occupies much less surface area in proportion to the area occupied by the total number of costae. But the asymmetry caused by odd and even numbers of costae on the fold can be appreciable.

ACKNOWLEDGEMENTS

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KUTCH MICROFAUNA—LOWER TERTIARY OSTRACODA

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ABSTRACT

Twenty-four new forms of Ostracoda including new species and varieties have been illustrated and described from the Middle Kirthar and Gaj beds of Kutch, Western India. Correlation of the marine Lower Tertiary beds of Western India is possible on the basis of the fossil Ostracode fauna.

INTRODUCTION

Except a few cursory remarks made on the occurrence of some Ostracode genera from the Tertiary rocks of India and Pakistan by Davies and Pinfold (1937), Jacob and Sastri (1952) and Tewari (1957), no detailed work has so far been published from India. A preliminary note on the Lower Miocene Ostracoda of Kutch, Western India has recently been published, (Tewari, Tandon and Bhargava, 1959) in which several genera belonging to the families Bairdiidae, Cypridae, Cytheridae and Cytherellidae have been recorded from the Lower and Upper Gaj beds.

Due to the lack of available literature on the Ostracode fauna from India and the neighbouring regions, most of the comparisons in the present study have been made with the European and American species.

The Ostracoda described in this paper come from the Lower Tertiary rocks of Vinjhan-Miani and Waghopadar-Cheropadi regions of south-western Kutch. The distance between the two areas is about 30 miles and they are separated by a tract of land covered by Recent and Sub-recent alluvium. The Lower Tertiary beds of Kutch have been sub-divided into Laki, Kirthar, Nari, Gaj, Post-Gaj and Manchar from bottom to top. Tewari (1956) indicated the age determinations of the beds as follows :

Manchar Middle Miocene to Pliocene
Post-Gaj Vindobonian ?
Gaj Upper Burdigalian
„ Lower Aquitanian
Nari Lattorian and/or Rupelian
Middle Kirthar Lutetian
Laki Ypresian

DESCRIPTION OF TYPE LOCALITIES

MIANI
(23°7'33" : 69°5'55")

Type Locality : Two furlongs north-west of the village Miani, on the left bank of the river Kankavati; surface sample number C5; locality I.

Lithology : Brownish yellow highly weathering marls, belonging to the lower part of the Middle Kirthars (Lutetian).

HEMRAI TALAO
(23°6'35" : 69°7'5")

Type Locality : One furlong north-west of Hemrai Talao, near the road leading to Hajapur; surface sample number C83; locality II.

Lithology : Yellowish brown, highly weathering marls, overlying mottled clays, belonging to the lower part of the Middle Kirthars (Lutetian).

VINJHAN
(23°6'10" : 69°4'50")

Type Locality : Half a mile north-east of the village Vinjhan on the road leading to Khirasra; surface sample number C14; locality III.

Lithology : Dark yellow argillaceous limestones with casts of Molluses, the basal members of the Upper Gaj (Burdigalian).

WAGHOPADAR
(23°28'40" : 68°47'25")

Type Locality : About half a mile north of Waghopadar from the plateau on the road leading to village Julera; surface sample number W5; locality IV.

Lithology : Cream coloured massive and thickly bedded limestone belonging to the lower part of the Middle Kirthars (Lutetian).

WAIOR
(23°25' : 68°44'20")

Type Locality : About three furlongs south of Waior in the Waior river section; surface sample number W18; locality V.

Lithology : Compact, brown foraminiferal marls intercalated with bands of shales containing fossil Algae and Foraminifera belonging to the upper part of the Lower Gaj beds (Aquitanian).

WAGHOT
(23°24' : 68°44')

Type Locality : Half a mile south of Waghoh in the Waior river section; surface sample number W25; locality VI.

Lithology : Cream coloured compact foraminiferal limestone, underlain by a band of shales containing white casts of *Turritella*, belonging to the Upper Gaj beds (Burdigalian).

CHEROPADI
(23°22'10" : 68°44'35")

Type Locality : Half a mile north of Cheropadi in the Waior river section; surface sample number W29; locality VII.

Lithology : Brownish yellow, easily weathering marls, underlain by shales belonging to the Upper Gaj beds (Burdigalian).

SYSTEMATIC DESCRIPTIONS

Order OSTRACODA Latreille, 1802

Suborder PODOCOPA Sars, 1865 (1866)

Family BAIRDIIDAE Sars, 1887

Subfamily BAIRDIINAE Sars, 1923

Genus BAIRDIA M'Coy, 1884

Bairdia indica Tewari and Tandon, n.sp.

Text-fig. 1, figure 1a-b

In side view subovoid, smooth and punctate carapace; left valve larger and overlapping the right valve. Dorsal margin arched, ventral margin pronouncedly convex anteriorly and slightly convex posteriorly. Highest portion of the carapace in middle. Anterior end of the dorsal side moderately sloping; posterior end steeper and forming an obtuse angle with the ventral side. In dorsal view carapace ovate, more compressed posteriorly than anteriorly; highly inflated anterior to middle, smooth and punctate.

Dimensions of the holotype no. 107, a complete carapace: length 1.12 mm., height 0.56 mm., width 0.44 mm.; paratype number 108, a complete carapace: length 1.01 mm., height 0.50 mm., width 0.40 mm.; paratype number 109, a complete carapace: length 1.11 mm., height 0.56 mm., width 0.44 mm.

Remarks: The present species differs from *Bairdia africana* in having a gentler slope on dorso-anterior side, an obtuse posterior angle and more inflated walls.

The species is commonly found in the beds of the Lutetian age occurring at localities I, II and IV.

Bairdia subdeltoidea var. *koteshwarensis*

Tewari and Tandon, n.var.

Text-fig. 1, figure 2a-b

Carapace elongate, ovate, subtriangular in side view; the larger left valve overlaps the right valve. Dorsal margin arched; ventral margin convex, the convexity is more towards the anterior side. The highest part of the carapace is in the middle. Anterior side broader than the posterior; broadly rounded in the ventral half and angularly rounded on the dorsal half. The posterior end is produced and narrow. The marginal area distinct with numerous radial canals. Carapace smooth and finely punctate. In dorsal view the widest part of the carapace in the middle; anterior and posterior ends compressed.

Dimensions of the holotype number 110, a complete carapace: length 1.08 mm., height 0.62 mm., width 0.48 mm.; paratype number 111, a complete carapace: length 1.03 mm., height 0.59 mm., width 0.46 mm.

Remarks: The species resembles in shape to *Bairdia wauchulensis* Swain, described from the Oligocene of Florida, but differs from the latter in a much higher ratio of length to height. *Bairdia laevicula* Edwards described from the Miocene of Florida Panhandle resembles the present species in its dorsal outline but the ventral side of the two are quite dissimilar. *Bairdia ocalana* from the Eocene of Florida is more massive and tumid species than the present. The nearest approach to this species is *Bairdia subdeltoidea* Münster from which it differs in having lesser convexity at postero-ventral angle and higher ratio of height to length of the carapace.

The species is rarely represented in the beds of the Lutetian age occurring at locality IV.

Bairdia ? kirtharensis

Tewari and Tandon, n.sp.

Text-fig. 1, figure 4a-b

In side view carapace large, subtriangular, dorsal margin convex, ventral margin slightly concave in the middle but convex at both the ends. Both the anterior and posterior ends somewhat rounded. Left valve larger and overlapping the right valve. Highest part of the carapace in middle and surface smooth. Specimens are always found closed, hence internal structures not seen.

Dimensions of the holotype number 112, a complete carapace: length 1.50 mm., height 0.70 mm., width 0.50 mm.; paratype number 113, a complete carapace: length 1.35 mm., height 0.63 mm., width 0.46 mm.

Remarks : The present species resembles *Bairdia chipolensis* described by Puri from the Miocene of Florida Panhandle but differs from it in having a different length to height ratio. Moreover, *Bairdia chipolensis* has a straight ventral side while in the present species it is concave. *Bairdia ubaghsi* described by Veen from the Maestrichtian of South Limburg differs from this species in having a straight ventral side and in details of the dorsal margin.

The species is abundantly represented in the beds of the Lutetian age occurring at localities I, II, and IV.

Bairdia sp.

Text-fig. 1, figure 3a-c

In side view carapace elongate and ovate. Dorsal margin arched, ventral margin curved anteriorly and almost flat posteriorly. Anterior end rounded with no antero-dorsal angle. Dorso-posterior side sloping and forming angularity with ventral margin. Highest part of the carapace a little posterior to middle and surface smooth. Marginal area broad with radial canals.

Dimensions of a right valve : length 0.86 mm., height 0.51 mm., width 0.12 mm.

Remarks : It is a very rare form and is generally found badly preserved making specific identification difficult ; occurs in the beds of Lutetian age at locality IV.

Genus BAIRDOPPILATA Coryell, Sample and Jennings, 1935

Bairdoppilata rajnathi

Tewari and Tandon, n.sp.

Text-fig. 1, figure 5a-b

In side view carapace large, inflated subtriangular. Dorsal margin arched but its middle portion straight with concave anterior and posterior ends. Ventral margin concave in the middle but convex at both ends. The upper half of the anterior end angularly rounded, posterior end is drawn out to a point below the middle line. Left valve larger than right and highest part of the carapace in middle. Hinge line in the right valve consists of two crenulated teeth situated at its extremities. Surface smooth, marginal area broad with pore canals. A few small indistinct spines on the posterior end of carapace. In dorsal view anterior and posterior ends compressed.

Dimensions of the holotype number 118, a right valve : length 1.38 mm., height 0.7 mm.; paratype number 119, a complete carapace: length 0.82 mm., height 0.45 mm., width 0.32 mm., and paratype number 120, a right valve : length 1.13 mm., height 0.63 mm.

Remarks : The species resembles in outline to *Bairdoppilata triangulata* described by Puri from Ecphora and Cancellaria facies of the Choctawhatchee stage, Miocene of Florida. But it differs from the American species in the shape of the posterior end, which is much more drawn out in the present form. Similarly, the position of the teeth on the hinge of this species is intermediate between that of *Bairdoppilata triangulata* and *B. willisensis* described by Puri from Miocene of Florida.

The species is named in honour of Prof. Raj Nath of Banaras Hindu University.

The species is commonly found in the beds of Burdigalian age occurring at locality VI.

Genus BYTHOCYPRIS Brady, 1880

Bythocypris mianica Tewari and Tandon, n.sp.

Text-fig. 2, figure 1a-b.

In side view the carapace is kidney shaped, medium and fragile. Dorsal margin strongly convex particularly in the middle. Anterior and posterior ends

rounded. Left valve larger overlapping the right valve dorsally and ventrally but not so much at the ends. Anterior end somewhat longer than the posterior. The highest part of the carapace a little anterior to the middle and surface smooth. In dorsal view it is oval in outline, anterior end somewhat less rounded than the posterior.

Dimensions of the holotype number 121, a complete carapace : length 1.22 mm., height 0.56 mm., width 0.48 mm.; paratype number 122, a complete carapace : length 1.20 mm., height 0.54 mm., width 0.45 mm.

Remarks : The species resembles *Bythocypris goodlandensis* Alexander described from the Albian of Texas in lateral view but is distinct from it in dorsal view in having both the ends comparatively more rounded. It resembles *Bythocypris parilis* Ulrich illustrated and described by Schmidt from the Eocene of Virginia, but differs from it in having the anterior side longer than the posterior, which is just the reverse of what we find in *Bythocypris parilis*.

The species is abundantly found in the beds of Lutetian age occurring at localities I, II and IV.

Family CYPRIDAE Baird, 1846

Genus PARACYPRIS Sars, 1866

Paracypris wynnei Tewari and Tandon, n.sp.

Text-fig.2, figure 2a-b

Carapace large, elongate, in side view and strongly tapering posteriorly. Anterior end obliquely rounded and strongly acuminate postero-ventrally. Dorsal margin moderately curved sloping gently anteriorly and posteriorly. Surface smooth and punctate. Larger left valve with a groove on the dorsal side to receive the dorsal edge of the right valve. Carapace highest a little anterior to the middle region with distinct radial canals. In dorsal view carapace highly elongated with anterior and posterior ends compressed.

Dimensions of the holotype number 123, a complete carapace : length 1.38 mm., height 0.46 mm., width 0.32 mm.; paratype number 124, a complete carapace : length 1.34 mm., height 0.41 mm., width 0.37 mm.

Remarks : The species closely resembles *Paracypris franquesi* Howe and Chambers redescribed by Stephenson from the Eocene rocks of Smithville, Texas from which it can be distinguished by its subcentral highest region, a slightly more obtuse posterior angle and the flat ventral side. It is also distinct from *Paracypris pacifiens* described from the recent sediments of Avalon Bay, Santa Catalina Island, which is characterised by its concavity of the ventral side and sharply pointed but slightly upturned posterior extremity.

The species is named in honour of A. B. Wynne, the pioneer geological worker of Kutch.

The species is rarely represented in the beds of Lutetian age occurring at locality IV.

Paracypris gajensis

Tewari and Tandon, n.sp.

Text-fig.2, figure 3a-b

In side view, the carapace large, elongate, sub triangular and lenticular. Anterior end broadly and evenly rounded, postero-ventrally acuminate and sharp. The dorsal margin angled and arched in well developed specimens. The posterior margin suddenly takes a turn and makes an angle with the ventral margin, which is almost straight. Highest portion of the carapace a little in front of middle. Larger left valve overlapping along the dorsal and middle of ventral margins, with a groove on dorsal margin to receive the sharp edge of the right valve. Marginal area broad with straight radial canals, anterior and posterior vestibules visible; surface smooth and punctate.

Dimensions of the holotype number 125, a complete carapace : length 1.34 mm., height 0.58 mm., width 0.26 mm.; paratype number 126, a complete carapace : length 1.2 mm., height 0.58 mm., width 0.40 mm.

Remarks : The species differs from *Paracypris choctawhatcheensis* Puri, 1953 from the Miocene of Florida Panhandle in having more convex and angular dorsal margin and straight ventral margin, which is gently concave in the former. The posterior end with its angularity is typical of this species and makes it distinct from *Paracypris wynnei* n.sp. and *Paracypris chipolensis* described from the Chipola facies of Miocene of Florida Panhandle. *Paracypris stolki* van den Bold, 1958 from Oligo-Miocene of Trinidad resembles this species in the shape of the anterior end but differs in the details of the shape of dorsal margin and postero-ventral angle. This species also differs from *Paracypris* aff. *limburgensis* and *Paracypris communis* from the Palaeocene of Trinidad in side view as well as in the posterior end.

The species is rarely represented in beds of the Lower Miocene age occurring at localities V and VI.

Family CYTHERIDAE Baird, 1850

Subfamily CYTHERIDEINAE Sars, 1925

Genus HAPLOCYTHERIDEA Stephenson, 1936

Haplocytheridea saurashtrensis

Tewari and Tandon, n.sp.

Text-fig. 6, figure 4a-c

In side view carapace elongate and oblong; anterior end broadly rounded, posterior end narrow but rounded. Dorsal margin moderately arched, ventral margin almost straight with concavity in front of the middle. Larger left valve overlapping the right at dorsal, posterior and ventral margins. It shows strong dimorphism, the females are more inflated and oblong. The hinge in the right valve consists of two crenulated teeth which fit into the corresponding crenulated grooves at the ends of the hinge in the left valve. Carapace concave ventrally in males but convex in females. Marginal area broad with indistinct pore canals; antero-ventral margin crenulated and posteriorly, the females are highly inflated. In dorsal view the carapace is widest, a little anterior to the middle.

Dimensions of the holotype number 127, a complete male carapace : length 0.71 mm., height 0.41 mm., width 0.36 mm.; paratype number 128, a female left valve : length 0.72 mm., height 0.45 mm.; paratype number 129, a male right valve : length 0.70 mm., height 0.38 mm.; paratype number 130, a left female valve : length 0.72 mm., height 0.45 mm.

Remarks : This species somewhat resembles *Haplocytheridea subovata* (Ulrich and Bassler) redescribed by van den Bold from the Oligo-Miocene of Trinidad but differs from it in having pitted surface. It also differs from *Haplocytheridea nowotnyi* described by Stephenson from the Eocene of Smithville, in the outline of carapace.

The species is abundantly represented in the beds of Aquitanian and Burdigalian ages occurring at localities III, V, VI and VII.

Genus KRITHE Brady, Crosskey and Robertson, 1874

Krithe indica

Tewari and Tandon, n.sp.

Text-fig. 2, figure 5a-b

In side view carapace elongate, smooth and obliquely truncate at the posterior end. Left valve overlapping the right. Anterior end broadly rounded, postero-ventral corner angulated. Highest portion a little posterior to the middle. Posterior end gently starts sloping from the middle.

Dimensions of the holotype number 131, a complete carapace : length 0.57 mm.; height 0.27 mm., width 0.28 mm.; paratype number 132, a complete carapace : length 0.55 mm., height 0.27 mm., width 0.27 mm.

Remarks : This species differs from *Krithe trinidadensis* in being less elongate but somewhat resembles *Krithe reversa* van den Bold from Oligo-Miocene of Trinidad in the posterior end of the carapace. *Krithe guatemulensis* van den Bold, from the Palaeocene of Trinidad is quite distinct in both the lateral and dorsal views.

The species is commonly represented in the beds of Lutetian age occurring at localities I, II and IV.

Krithe indica var. *kutchensis*
Tewari and Tandon, n.sp. and n.var.
Text-fig. 6, figure 2a-b

This variety is similar to *Krithe indica* but differs slightly in the slope on the posterior side which in this case is abrupt and large. Moreover, *Krithe indica* is more compressed on the posterior side. Marginal area broad. Internal characters are not seen as only closed carapace are found.

Dimensions of the holotype number 133, a complete carapace : length 0.80 mm., height 0.36 mm., width 0.36 mm.; paratype number 134, a complete carapace : length 0.55 mm., height 0.25 mm., width 0.24 mm.

The species is commonly represented in the beds of Aquitanian and Burdigalian ages occurring at localities III, V and VI.

Subfamily PARACYTHERIDINAE, Puri, 1957
Genus PARACYTHERIDEA G. W. Müller, 1894

Paracytheridea misrai
Tewari and Tandon n.sp.
Text-fig. 2, figure 4a-c

In side view carapace subquadrate. upper part of the anterior end obliquely rounded, lower part broadly rounded, dorsal margin straight and ventral margin somewhat sinuous. Posterior end truncated and produced in an angularity. Surface smooth with a prominent ventral edge and backwardly directed alae form ridge which ends in a spine. Swelling on the posterior cardinal angle with an oblique ridge and also on the antero-ventral part of the carapace with two sinuous fine ridges. Eyespot indistinct. Hinge in the right valve almost straight with weakly developed crenulated teeth at both ends of the hinge. Marginal area moderately broad and marginal pore canals obscure. In the dorsal view carapace compressed at both anterior and posterior ends with maximum thickness behind middle.

Dimensions of the holotype number 136, a right valve : length 0.67 mm., height 0.33 mm., width 0.28 mm.; paratype number 137, a right valve : length 0.66 mm., height 0.33 mm., width 0.28 mm.

Remarks : The species is quite distinct in shape and ornamentation from *Paracytheridea tschoppi* described by van den Bold from Oligo-Miocene of the Southern Trinidad. It also differs from *Paracytheridea chipolensis* in outline and ornamentation of carapace. In side view the species has some resemblance to *Paracytheridea washingtonensis* but it differs from the latter species in having a more inflated carapace, longer alae form spine and characteristic ornamentation of the carapace.

The species is named in honour of Prof. R. C. Misra of Lucknow University.

The species is rarely represented in the beds of Aquitanian age occurring at locality V.

Subfamily TRACHYLEBERINAE Sylvester-Bradley, 1948

Genus ACTINOCYTHEREIS Puri, 1953

Actinocythereis gujaratensis

Tewari and Tandon, n.sp.

Text-fig. 3, figure 1a-b

Carapace stout, subrhomboidal in side view, anterior end obliquely rounded, posterior end round; both the ends crenulated. Dorsal margin jagged, ventral margin almost straight, anterior marginal rim broad and elevated. Surface with coarse ornamentation of a few large knobs, occasionally elongated, transverse to the length of the carapace and arranged in three rows along the length of the carapace. Hinge line straight, holamphidont, consisting of an anterior stepped tooth, a postjacent socket followed by a groove and posterior rounded tooth. Anterior marginal area broad and with numerous pore canals.

Dimensions of the holotype number 138, a right valve: length 0.68 mm., height 0.35 mm.; paratype number 139, a right valve: length 0.65 mm., height 0.33 mm.

Remarks: This species differs from *Actinocythereis howei* Marianos and Valentine from the Eocene of California in details of shape, with which it has slight resemblance in ornamentation. *Actinocythereis exanthemata* seems to have close resemblance in outline of carapace to the present species. However, it can be distinguished from the latter by its coarse ornamentation.

The species is commonly represented in the beds of Aquitanian age occurring at locality V and in the beds of the Burdigalian age occurring at locality VII.

Actinocythereis uniorensis

Tewari and Tandon, n.sp.

Text-fig.3, figure 2a-b

In side view carapace subquadrate and stout; anterior end obliquely rounded, posterior end broadly rounded. Dorsal and ventral lines almost straight and parallel to each other. Anterior marginal rim broad, elevated with small nodules on its surface. Both the anterior and posterior ends crenulated. Surface covered by a few small nodules arranged in three rows parallel to the length of the carapace; posterior end has spiny nodules. Hinge straight, holamphidont, marginal area broad with few pore canals.

Dimensions of the holotype number 140, a complete right valve: length 1.04 mm., height 0.48 mm.; paratype number 141, a complete left valve: length 0.95 mm., height 0.43 mm.

Remarks: This species can be distinguished from *Actinocythereis gujaratensis* n.sp. by fewer elements of ornamentation and the outline of carapace.

The species is commonly represented in the beds of Aquitanian age occurring at locality V.

Genus PTERYGOCYTHEREIS Blake, 1933

Pterygocythereis marhensis

Tewari and Tandon n.sp.

Text-fig.3, figure 3a-c

In side view carapace subquadrate, elongate; anterior end broadly rounded and posterior end somewhat truncated and pointed. Dorsal margin straight, ventral margin almost straight; eyespot distinct. An alae form ridge on the ventral side, directed posteriorly; surface reticulated. As seen from the inner side the carapace is moderately deep. Hinge almost straight which is typically holamphidont.

In the right valve there is an anterior stepped tooth, a postjacent socket connected with a bar. Anterior marginal area broad, pore canals indistinct. In dorsal view both anterior and posterior ends compressed; indistinct portion on the posterior end of the ala.

Dimensions of the holotype number 142, a complete right valve: length 0.94 mm., height 0.57 mm.; paratype number 143, a complete left valve: length 0.93 mm., height 0.57 mm.

Remarks: *Pterygocythereis cornuta americana* somewhat resembles this species in outline of the carapace but differs in having a smooth surface and presence of coarse crenulations on the posterior side. *Pterygocythereis* sp. described by van den Bold from the Palaeocene of Trinidad has a smaller ventral ala, with another weaker, thinner ridge below and parallel to it and surface comparatively smooth. Present species is characterised by a prominent ventral alae and strong reticulation on the surface. Anterior end of carapace finely denticulate, while the posterior end has one or two blunt spines.

The species is rarely represented in the beds of Lutetian age occurring at locality I.

Genus TRACHYLEBERIS Brady, 1898

Trachyleberis ? bhujensis

Tewari and Tandon, n.sp.

Text-fig. 6, figure 3a-b

In side view carapace subquadrate, small and both right and left valves of equal size. Anterior end broadly rounded, posterior end narrowly rounded. Both the anterior and posterior marginal rims prominent; anterior side denticulate and posterior side with spiny processes. Ventral edge straight, dorsal margin jagged due to the presence of spines. Surface of the carapace covered by very fine spines. Eyespots prominent; hinge line straight. In dorsal view the carapace compressed both anteriorly as well as posteriorly; widest part in the middle region.

Dimension of the holotype number 144, a complete carapace: length 0.55 mm., height 0.28 mm., width 0.20 mm.; paratype number 145, a complete carapace: length 0.73 mm., height 0.33 mm., width 0.23 mm.

Remarks: The species resembles *Trachyleberis ? grigsbyi* (Howe and Chambers) in shape and outline of the carapace but differs in ornamentation. *Trachyleberis ? splendens* is similar in ornamentation and in the presence of a prominent eyespot but is quite dissimilar in shape as compared to the present species.

The species is rarely represented in the beds of Lutetian age occurring at locality I.

Trachyleberis vinjhanensis

Tewari and Tandon, n. sp.

Text-fig.3, figure 4a-b

In side view carapace elongate, subquadrate, heavy, lower half of the anterior end broadly rounded, upper half obliquely rounded; posterior end obtuse; highest portion at the anterior cardinal angle; dorsal and ventral margins jagged. Anterior end finely and posterior end coarsely crenulate. Anterior marginal rim broad and elevated with nodes on its surface. Surface of carapace covered by large and small irregularly distributed nodes. Hinge straight, and in the right valve consists of an anterior rounded tooth, a postjacent socket and a posterior rounded tooth connected by a groove which becomes broader in the posterior end. Marginal area broad with pore canals.

Dimensions of the holotype number 146, a complete right valve: length 1.00 mm., height 0.52 mm.; paratype number 147, a complete right valve: length 0.95 mm., height 0.48 mm.

Remarks : The species differs from *Trachyleberis montgomeryensis* (Howe and Chambers) in having knobs instead of spines on the surface as found in the latter species. It also differs from *Trachyleberis scabrocuneata* (Brady) in the shape and ornamentation of the carapace. *Trachyleberis bhujensis* n.sp. from Kutch is a smaller form with more converging dorsal and ventral margins and very fine ornamentation of small spines, but differs from the latter in ornamentation. It resembles feebly to *Trachyleberis paucispinata*, Marianos and Valentine in the posterior end of carapace.

The species is abundantly represented in the beds of Aquitanian and Burdigalian ages occurring at localities III, V, VI and VII.

Genus JUGOSOCYHEREIS Puri, 1957

Jugosocythereis ? sp.

Text-fig. 3, figure 5

In side view, carapace elongate and subquadrate. Anterior end broadly rounded in the lower part and obliquely rounded in the upper part. Dorsal margin nearly straight on posterior end but gently concave in the anterior part; ventral margin almost straight. Highest part of the carapace at the anterior cardinal angle. Posterior end compressed, truncated and angular near the ventral margin. Anterior end very finely denticulate; anterior marginal rim narrow and distinct. Subtriangular posterior end with three or four small blunt projections. Carapace ornamented with two prominent ridges somewhat elevated near the posterior end, the one on the posterior cardinal angle slightly curved. There are fine longitudinal ridges all over the surface and the area between them is pitted. There is a small sub-central tubercle; eyespot distinct.

Dimensions of the specimen number 148, a complete carapace : length 0.76 mm., height 0.41 mm., width 0.31 mm.

Remarks : A solitary closed carapace has been found which prevents us from assigning it a specific name. However, the species resembles in outline of the carapace to *Jugosocythereis tricarinata* described by Puri from Crystal river formation, Florida from which it can be distinguished by its characteristic ornamentation.

The species is rarely found in the beds of Burdigalian age occurring at locality VI.

Genus CYHEREIS Jones, 1849

Cythereis ? *kankaratiensis*

Tewari and Tandon, n.sp.

Text-fig. 5, figure 3

In side view carapace subquadrate, stout; dorsal and ventral margins straight. Anterior end broadly rounded and posterior end attenuated and obtuse. Anterior marginal rim present, posterior rim very distinct and surface ornamented with coarse reticulation. The characteristic feature of the species is the inflated wall a little posterior to the centre and coarse reticulation. Eyespot and muscle scar indistinct. Maximum height near the anterior cardinal angle.

Dimensions of the holotype number 149, a complete carapace ; length 0.77 mm., height 0.37 mm., width 0.27 mm.; paratype number 150, a complete carapace : length 0.78 mm., height 0.37 mm., width 0.28 mm.

Remarks : This species resembles in ornamentation with *Cythereis bursilloides* Stadnichenko redescribed by Stephenson from Eocene of Smithville, Texas but differs in the shape of the posterior region, which is somewhat rounded in the latter species but is truncated in the present species.

The species is commonly represented in the beds of Lutetian age occurring at localities I, II and IV.

Subfamily HEMICYTHERINAE Puri, 1953

Genus HEMICYTHERE Sars, 1925

Hemicythere sahnii

Tewari and Tandon, n.sp.

Text-fig. 4, figure 1a-d

In side view the carapace large, subquadrate, left valve larger than the right; highest point near the eyespot. Anterior end broad and obliquely rounded; posterior end lower, bluntly angular. Wall of the carapace inflated ventrally with a ventral alae form expansion with two longitudinal ridges which die out posteriorly. Eyespot prominent. Dorsal margin moderately arched, sloping posteriorly. Ventral margin somewhat flat with a characteristic antero-ventral angle. Surface of the carapace smooth with a depression on the ventral line and little anterior to the middle representing inturned ventral margin. Hinge curved, in the left valve it consists of an anterior rounded socket, a postjacent smooth tooth, followed by a narrow bar to the posterior rounded socket. Right valve is complimentary with the anterior socket and posterior noncrenulate socket of the left. The ridge or bar is separated from the dorsal margin by a narrow furrow; marginal areas are fairly broad with numerous radial canals. In dorsal view the anterior and posterior ends compressed.

Dimensions of the holotype number 151, a complete carapace : length 0.85 mm., height 0.48 mm., width 0.40 mm.; paratype number 152, a left valve : length 0.73 mm., and height 0.43 mm.

Remarks : This species differs from *Hemicythere amygdala* described by Stephenson from Middle Tertiary strata of Texas in the shape of carapace and smooth surface which is pitted in the latter. There is also absence of alae in the species of Texas. It also resembles in outline with *Hemicythere punctata*, described by Puri from the Upper Eocene beds of Crystal river formation from which it differs in having a smooth carapace instead of finely pitted ornamentation of *Hemicythere punctata*. The posterior end of the present species is dissimilar from it. Present species is characterised by the presence of two longitudinal ridges on the ventral alae form expansion which are absent in *Hemicythere amygdala*.

The species is named in honour of Dr. M. R. Sahni.

It is abundantly represented in the beds of Lutetian age occurring at localities I, II and IV.

Hemicythere aff. *amygdala* Stephenson

Text-fig. 4, figure 2a-b

Hemicythere amygdala Stephenson, 1944, *J. Palaeont.*, **18**, p. 158, pl. 28, figures 8, 9.

Hemicythere amygdala Stephenson, Puri, 1953-b, *J. Wash. Acad. Sci.*, **43**, No. 6, p. 176, pl. 1, figure 3.

Hemicythere amygdala Stephenson, Puri, 1953, *Bull. Fla. geol. Surv.*, No. 36, p. 266, pl. 14, figure 11.

In lateral view carapace of medium size and almond shaped with upper half of posterior end concave, lower half obtuse. Highest portion of carapace in middle; ventral margin slightly convex. Lower part of the anterior end rounded, upper part obliquely rounded. Valves of equal size with reticulated or pitted surface. The hinge of the right valve with a knob like anterior tooth, a broad postjacent smooth socket followed by a groove and a rounded prominent tooth at the posterior end. Marginal area broad, pore canals numerous and straight. Carapace inflated near the ventral side with two longitudinal ridges. In dorsal view the widest part of the carapace just posterior to middle.

Dimensions of the specimen number 153, a right valve : length 0.70 mm., height 0.45 mm.; specimen number 154, a right valve : length 0.64 mm., height 0.49 mm.

Remarks : This species has close resemblance to *Hemicythere amygdala* described from the Chipola facies of the Miocene of Florida; nevertheless, it has minor differences with the latter in details of posterior end.

It is abundantly represented in the beds of Aquitanian and Burdigalian ages occurring at localities III, V, VI and VII.

Genus HERMANIA Puri, 1953

Hermania indica

Tewari and Tandon, n.sp.

Text-fig. 4, figure 3a-c

In side view carapace subquadrate and elongate. Anterior side broadly rounded and crenulated; posterior end angular with few spines. Ventral margin moderately convex on the posterior side; a ridge on the ventral side of the carapace starting suddenly from the posterior side and gradually dying out at the anterior end. Anterior cardinal angle well developed. Surface of the carapace pitted and covered with small rounded nodules. A pronounced subcentral muscle-scar, node and eyespot visible. Anterior and posterior marginal rims narrow. Internally the dorsal line is irregular; hinge line is straight and in the left valve consists of an anterior socket, a postjacent rounded tooth followed by a finely crenulated bar and a posterior rounded socket. Greatest height of the carapace at the anterior cardinal angle, anterior and posterior ends compressed. Marginal area broad and radial canals present.

Dimensions of the holotype number 155, a complete carapace : length 0.83 mm., height 0.46 mm., width 0.45 mm.; paratype number 156, a left valve : length 0.73 mm., height 0.43 mm.

Remarks : As seen from the ventral side *Hermania reticulata* Puri, 1953 described from the Chipola facies of the Miocene of Florida, closely resembles the present species. However, in lateral view the present species is quite distinct from *Hermania reticulata* in having dorsal margin more pronouncedly sloping and sub-central muscle scar node produced in the form of a ridge anteriorly. The anterior cardinal angle is more pronounced and the dorsal keel fairly well developed.

The species is abundantly found in the beds of Lutetian age occurring at localities I, II and IV.

Hermania purii

Tewari and Tandon, n.sp.

Text-fig. 5, figure 1a-b

In side view carapace subquadrate, left valve moderately overlapping the right, anterior to middle; anterior end obliquely rounded, posterior end somewhat produced with five spiny processes. Dorsal line slightly wavy, ventral line undulating. Anterior marginal rim distinct. Highest part of the carapace at the anterior cardinal angle; surface reticulate. There is a ventral ridge starting one-fourth distance from the posterior end gradually disappearing at the antero-ventral angle; a faint dorsal ridge starting from one-fourth of the posterior end disappearing at the antero-cardinal angle with a break in the middle of the dorsal line. Both the ridges are alate. Hinge on the left valve, consists of a large smooth anterior socket, a postjacent smooth tooth and a posterior rounded socket connected by a long bar which is slightly crenulated and straight. Eyespot and smooth sub-central postule prominent. In dorsal view maximum width of the carapace a little anterior to the middle; posterior end compressed. Marginal area broad with pore canals.

Dimensions of the holotype number 157, a complete left valve : length 0.66 mm., height 0.37 mm.; paratype number 158, a complete left valve: length 0.64 mm., and height 0.36 mm.

Remarks : The present species closely resembles *Hermania reticulata* described by Puri from Miocene of Florida Panhandle and occurring abundantly in the Chipola facies. However, there is a distinct difference between the two species with regard to the posterior end. In the former species the posterior end is sub-acute, truncated and produced; stuffed with six or seven ventrally projecting spines in the ventral half of the posterior end. In the present species, however, the posterior end is not much truncated and contains only five spines.

The species is named in honour of Dr. H. S. Puri of Florida Geological Survey, Tallahassee, Florida.

The species is commonly found in the beds of Aquitanian age occurring at locality V.

Subfamily CYTHERURINAE Müller, 1844

Genus PAIJENBORCHELLA Kingma, 1894

Paijenborchella boldi

Tewari and Tandon, n. sp.

Text-fig. 5, figure 2a-b

In side view carapace subquadrate, elongate. Dorsal margin sinuous; ventral margin somewhat concave. Anterior end obliquely rounded, posterior end truncated and produced in a caudal process at the base of ventral margin. Anterior marginal area broad and distinct. Highest part of the carapace at the anterior cardinal angle. A faint vertical sulcus in the middle of carapace; an alae form ridge starts from the junction of the posterior end and the caudal process, proceeds sinuously joining the anterior rim. Two hump like swellings near posterior cardinal angle connected with each other; two swellings in front of the middle near the ventral margin. The carapace has always been found closed, hence the internal characters not visible.

Dimensions of the holotype number 159, a complete carapace : length 0.85mm., height 0.41 mm., width 0.41 mm.; paratype number 160, a complete carapace : length 0.72 mm., height 0.38 mm. and width 0.37 mm.

Remarks : The species differs from *Paijenborchella marssoni* described from the Cretaceous of Rugen and *Paijenborchella trigona* described by Marianos and Valentine from the Eocene of California in details of shape and ornamentation of the carapace.

The species is named in honour of Dr. W. A. van den Bold of Bataafsche Petroleum Maatschappij, The Hague.

The species is commonly represented in the beds of Aquitanian age occurring at locality V.

Subfamily CYTHERETTINAE Triebel, 1952

Genus CYTHERETTA G. W. Müller, 1894

Cytheretta cheropadiensis

Tewari and Tandon, n.sp.

Text-fig. 5, figure 4a-b

In side view carapace elongate and ovate. Dorsal and ventral margin almost parallel, dorsal margin slightly curved. Anterior end broadly rounded; anterior cardinal angle prominent and posterior distinct. Anterior marginal rim broad, elevated and smooth. Left valve larger, overlapping the right valve at the cardinal angles and in the middle of ventral margin. Hinge line straight and in the left valve consists of a prominent anterior socket, a postjacent tooth followed by a bar and on the posterior end there is a shallow socket. In the right valve, the hinge is

complementary with the anterior smooth socket of the left valve. Highest part of the carapace near the anterior cardinal angle. Surface pitted, marginal area broad with pore canals.

Dimensions of the holotype number 161, a complete left valve : length 0.85 mm., height 0.48 mm.; paratype number 162, a complete left valve : length 0.88 mm., and height 0.50 mm.

Remarks : This species differs from *Cytheretta alexanderi* Howe and Chambers redescribed by Blake from the Gosport sand of the Claiborne Eocene, Clarke County, Alabama, in the shape of the carapace as well as in the ornamentation. *Cytheretta burnsi* (Ulrich and Bassler), redescribed by Puri from the Middle Miocene Area zone of the Choctawhatchee formation of Florida, differs from the present species in ornamentation and in the absence of any prominent anterior marginal rim.

The species is commonly represented in the beds of Aquitanian and Burdigalian ages occurring at localities V, VI and VII.

Suborder PLATYCOPA Sars, 1866

Family CYTHERELLIDAE Sars, 1866

Genus CYTHERELLOIDEA Alexander, 1929

Cytherelloidea kathiawarensis

Tewari and Tandon, n.sp.

Text-fig. 5, figure 5

In side view carapace thick, subquadrate and oblong. Dorsal and ventral margins straight and almost parallel to each other. Highest part of the carapace at the anterior cardinal angle. Both the anterior and posterior ends broadly rounded and smooth. Posterior end narrower. flat carapace, surface ornamented by a marginal ridge continuous all round it and widest at the antero-ventral angle. Another ridge starts from the circum-marginal ridge, a little below the middle at the posterior end and extends towards the antero-cardinal angle terminating in a downward slope within the outer ridge. Surface punctate and smooth. In dorsal view anterior end rounded and posterior end compressed.

Dimensions of the holotype number 163, a complete carapace : length 0.61 mm., height 0.32 mm., width 0.24 mm.; paratype number 164, a complete carapace : length 0.59 mm., height 0.30 mm., and width 0.22 mm.

Remarks : The present species resembles *Cytherelloidea leonensis*, Howe (1928) from the Miocene of Gulf Coast but differs mainly in the length of the inner ridge which in the present species extends up to three-fourth of the carapace, whereas, in the former species it extends only up to one-fourth of the length of carapace. Moreover, the inner ridge in this species is oblique to the outer ridge but in case of *Cytherelloidea leonensis*, it is parallel. The present species also differs in the details of outline of the carapace with *Cytherelloidea denticulata* (Bosquet) described from the Maestrichtian of Limburg. It somewhat resembles in outline of the carapace to *Cytherelloidea subgoodlandensis* Wanderpool, 1933, but completely differs in ornamentation since the latter has two longitudinal ridges within circum-marginal ridge.

The species is rarely represented in the beds of Burdigalian age occurring at locality VI.

Cytherelloidea barkhanensis

Tewari and Tandon, n.sp.

Text-fig. 5, figure 6a-b

Text-fig. 6, figure 1

In side view carapace small, oblong; showing clear dimorphism. Dorsal margin depressed and wavy, ventral margin slightly curved, particularly in the antero-

ventral and postero-ventral regions. Anterior end broadly rounded and posterior end nearly so. Marginal ridge all round the periphery, almost following the shape of the carapace. Surface smooth and punctate. The carapace in female larger and more inflated posteriorly than in the male. Two swellings on the posterior side of the marginal ridge and inside the carapace represented by two depressions.

Dimensions of the holotype number 165, a complete female right valve : length 0.63 mm., height 0.35 mm.; holotype number 166, a male right valve : length 0.58 mm., height 0.30 mm.; paratype number 167, a female right valve : length 0.61 mm., height 0.33 mm.; paratype number 168, a male right valve : length 0.57 mm., height 0.29 mm.

Remarks : The species resembles *Cytherelloidea williamsonia* in the shape of the anterior side but posterior ends of both the species are quite different. *Cytherelloidea leonensis* Howe, redescribed by Puri from Choctawhatchee stage of the Miocene of Florida resembles somewhat in the marginal ridge but is quite distinct in the shape of the carapace. *Cytherelloidea denticulata* from the Maestrichtian of Limburg is distinct from the present species in shape of the dorsal and ventral margins and is characterised by the rectangular posterior end.

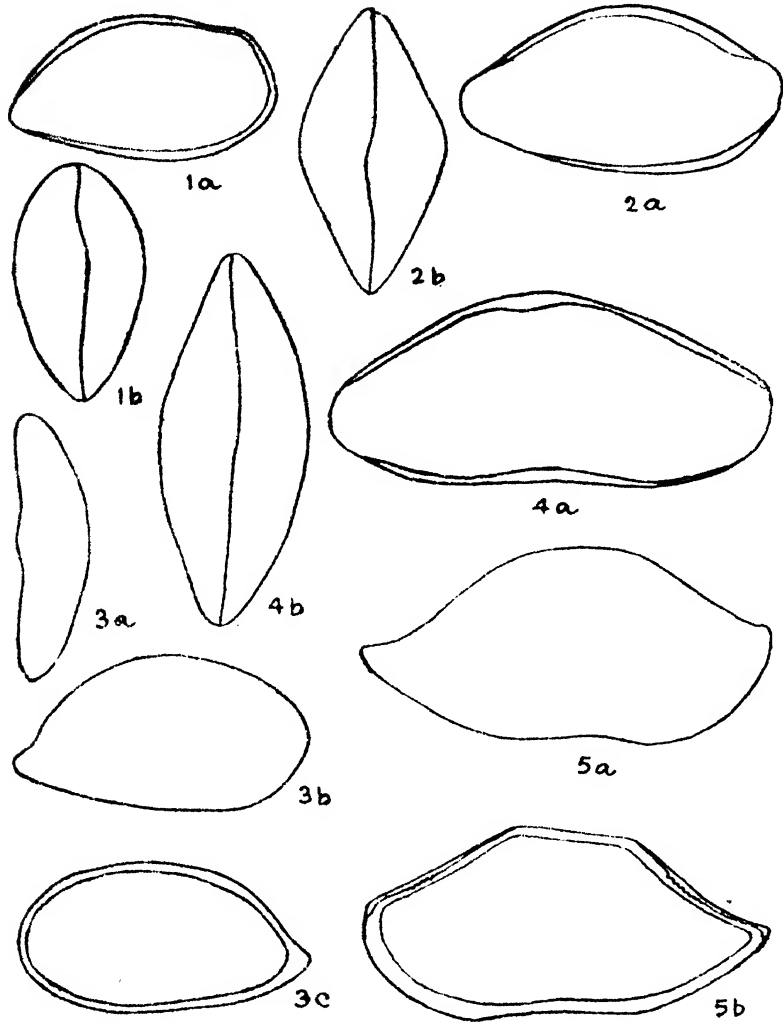
The species is abundantly represented in the beds of Aquitanian and Burdigalian ages occurring at localities III, V, VI and VII.

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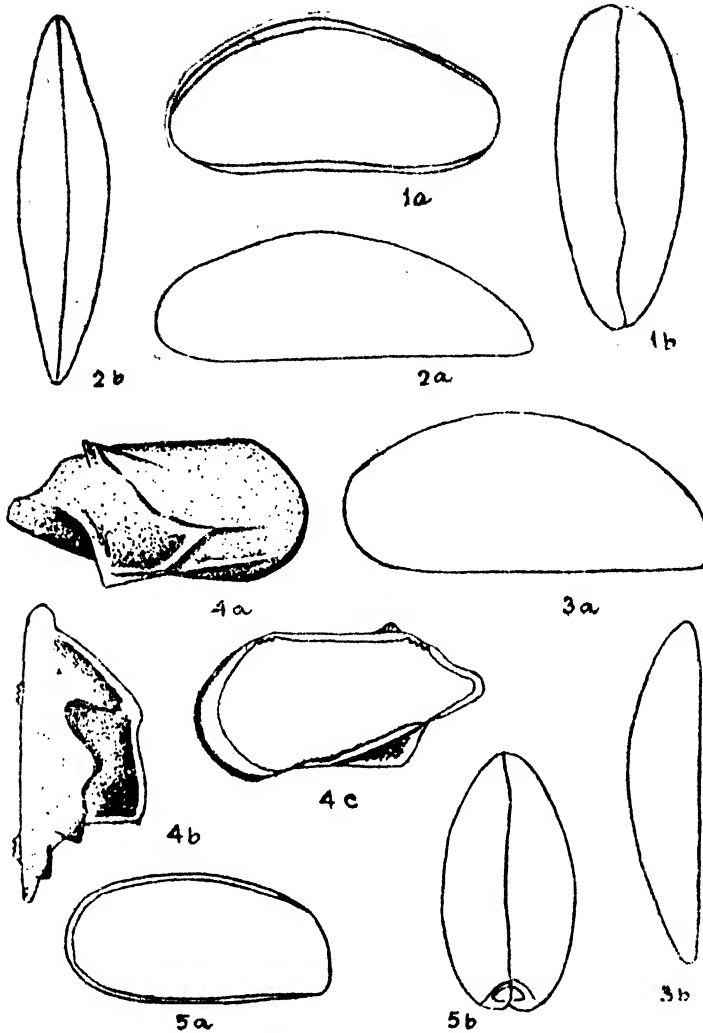
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TEXT-FIG. 1.

Magnification about $34\times$

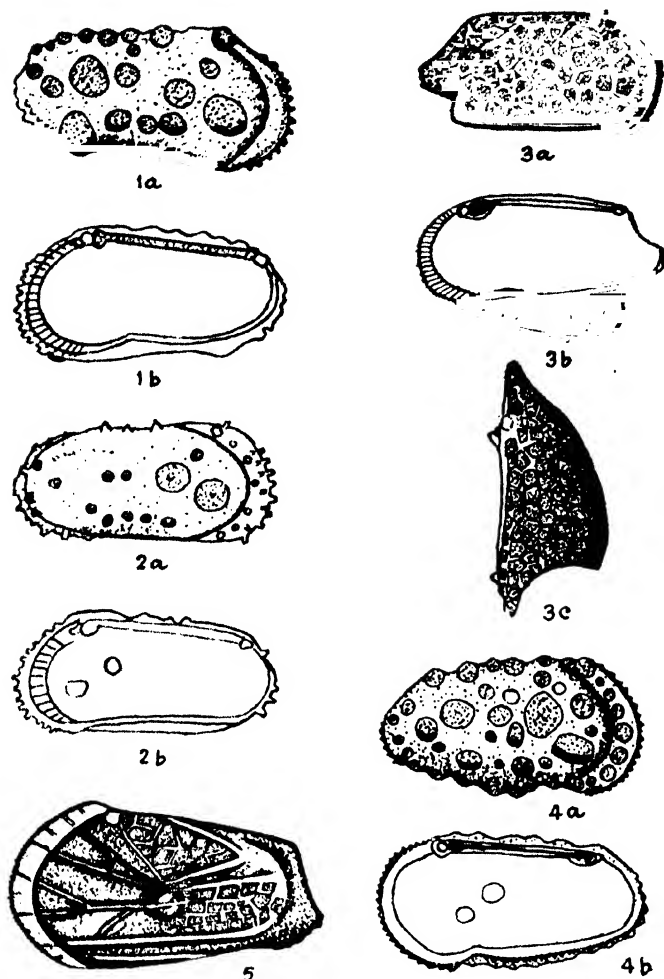
- Fig. 1. *Bairdia indica* Tewari and Tandon, n.sp.
Complete carapace: a, right valve view; b, dorsal view.
- Fig. 2. *Bairdia subdelloidea* var. *koteswarensis* Tewari and Tandon, n.var.
Complete carapace: a, right valve view; b, dorsal view.
- Fig. 3. *Bairdia* sp.
Right valve: a, dorsal view; b, external view; c, internal view.
- Fig. 4. *Bairdia? kirtharensis* Tewari and Tandon, n.sp.
Complete carapace: a, right valve view; b, dorsal view.
- Fig. 5. *Bairdoppilata rajnathi* Tewari and Tandon, n.sp.
Right valve: a, external view; b, internal view.



TEXT-FIG. 2.

Magnification about $34 \times$ unless otherwise indicated

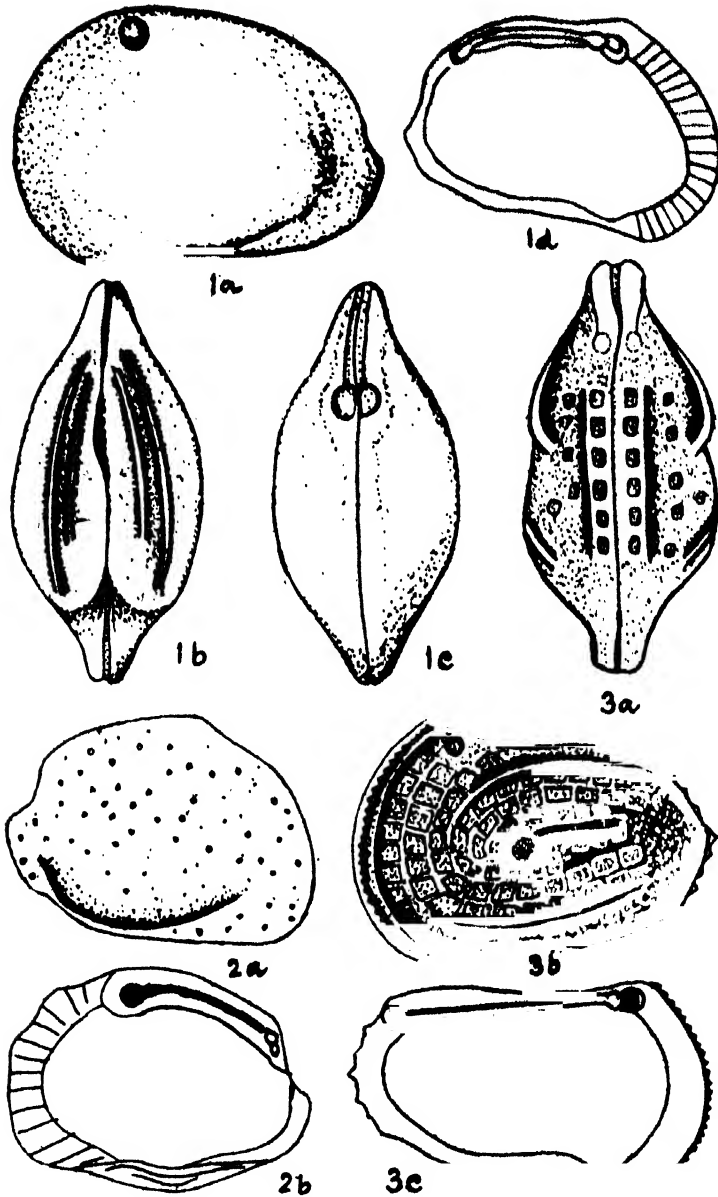
- Fig. 1. *Bythocypris mianica* Tewari and Tandon, n.sp.
Complete carapace : a, right valve view; b, dorsal view.
- Fig. 2. *Paracypris wyntei* Tewari and Tandon, n.sp.
Complete carapace : a, left valve view; b, dorsal view.
- Fig. 3. *Paracypris gajensis* Tewari and Tandon, n.sp.
Left valve : a, external view; b, dorsal view.
- Fig. 4. *Paracytheridea misrai* Tewari and Tandon, n.sp.
Right valve, \times about 54 : a, external view; b, dorsal view; c, internal view.
- Fig. 5. *Kriethe indica* Tewari and Tandon, n.sp.
Complete carapace, \times about 54 : a, left valve view; b, dorsal view.



TEXT-FIG. 3.

Magnification about 34 \times unless otherwise indicated

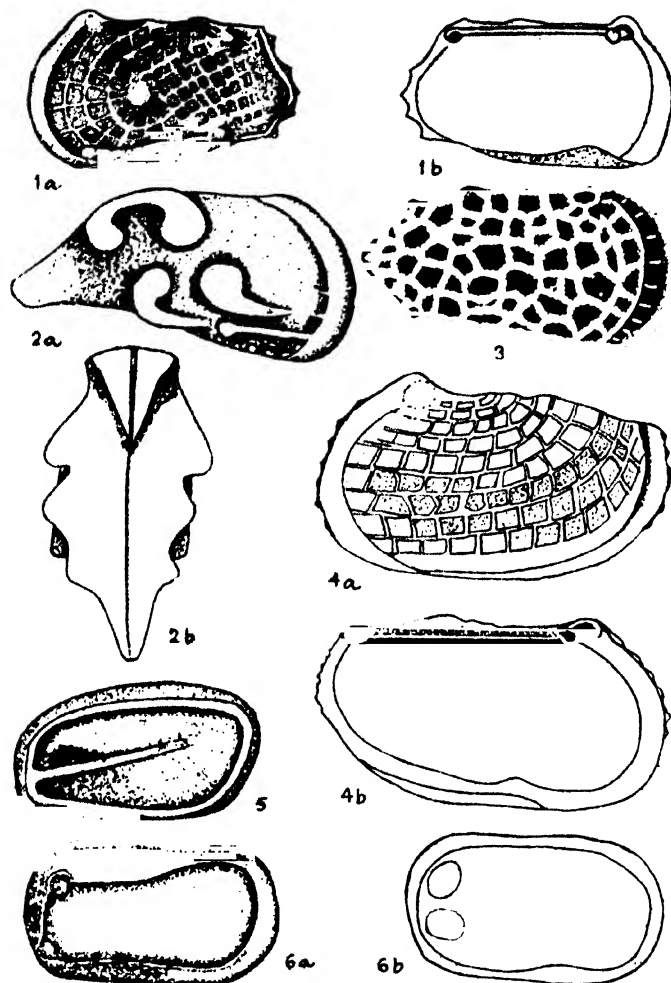
- Fig. 1. *Actinocythereis gujaratensis* Tewari and Tandon, n.sp.
Right valve: a, external view; b, internal view.
- Fig. 2. *Actinocythereis waiorensis* Tewari and Tandon, n.sp.
Right valve: a, external view; b, internal view.
- Fig. 3. *Pterygocythereis marhensis* Tewari and Tandon, n.sp.
Right valve: a, external view; b, internal view; c, dorsal view.
- Fig. 4. *Trachyleberis vinjhanensis* Tewari and Tandon, n.sp.
Right valve: a, external view; b, internal view.
- Fig. 5. *Jugosocythereis* ? sp.
Complete carapace, \times about 54: left valve view.



TEXT-FIG. 4.

Magnification about 54×

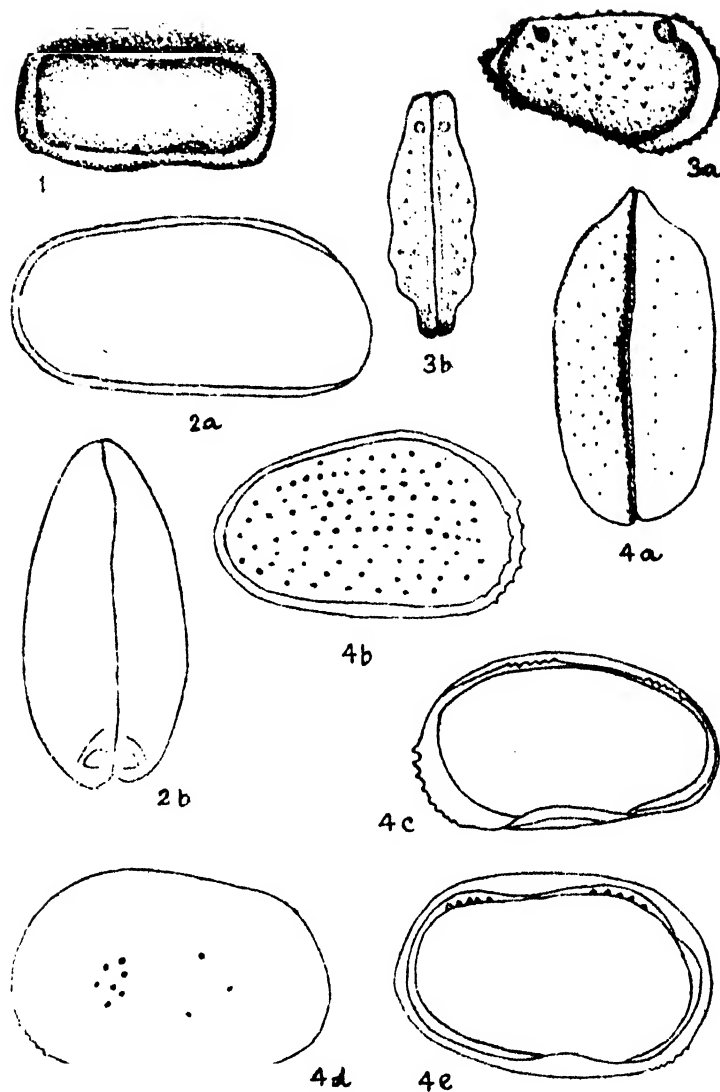
- Fig. 1. *Hemicythere sahnii* Towari and Tandon, n.sp.
a-c, complete carapace: a, left valve view; b, ventral view; c, dorsal view; d, internal view of left valve.
- Fig. 2. *Hemicythere* aff. *amygdala* Stephenson
Right valve: a, external view; b, internal view.
- Fig. 3. *Hermania indica* Towari and Tandon, n.sp.
a-b, complete carapace: a, dorsal view; b, left valve view; c, internal view of left valve.



TEXT-FIG. 5.

Magnification about 54 ×.

- Fig. 1. *Hermunia purii* Towari and Tandon, n.sp.
Left valve: a, external view; b, internal view.
- Fig. 2. *Puijenborchella boldi* Towari and Tandon, n.sp.
Complete carapace: a, right valve view; b, dorsal view.
- Fig. 3. *Cythereis? kankawatiensis* Towari and Tandon, n.sp.
Complete carapace: right valve view.
- Fig. 4. *Cytherella cheropadiensis* Towari and Tandon, n.sp.
Left valve: a, external view; b, internal view.
- Fig. 5. *Cytherelloidea kathiawarensis* Towari and Tandon, n.sp.
Complete carapace: right valve view.
- Fig. 6. *Cytherelloidea barkhanensis* Towari and Tandon, n.sp.
Female right valve: a, external view; b, internal view.



TEXT-FIG. 6.

Magnification about $54\times$

- Fig. 1. *Cytherelloidea barkhanensis* Tewari and Tandon, n.sp.
Male right valve: external view.
- Fig. 2. *Krithe indica* var. *kutchensis* Tewari and Tandon, n.sp. and n. var.
Complete carapace: a, left valve view; b, dorsal view.
- Fig. 3. *Trachyleberis ? bhujensis* Tewari and Tandon, n.sp.
Complete carapace: a, right valve view; b, dorsal view.
- Fig. 4. *Haplocytheridea saurashtrensis* Tewari and Tandon, n.sp.
a-b, male carapace: a, dorsal view; b, right valve view; c, male right valve, internal view; d-e, female left valve: d, left valve view; e, internal view.

SOME NEW SPECIES OF CHALCIDS FROM INDIA

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ABSTRACT

During the course of the preliminary survey of parasites of crop pests in the Indian Union the following parasites were reared. Two species of parasites belonging to the genera *Dasy-scapus* and *Tetrastichus* were reared from some thrips. Both the species were found to be new species and described as *Dasyscapus thripsivorous*, new species and *Tetrastichus rhipophora-thripacidia*, new species. Parasites from thrips are most uncommon. Two new species of *Anastatus* were also reared from the eggs of *Acherontia styx* and *Halis dentata*. These have been described as *Anastatus acherontiae*, new species and *Anastatus dentatus*, new species. Another species belonging to the genus *Closterocerus* was reared from *Agromyza* sp. and has been described as *Closterocerus agromyzae*, new species.

A species of the genus *Closterocerus* has been described for the first time from India. A key to Indian species of *Anastatus* has been given.

INTRODUCTION

During the course of the survey of beneficial parasites and predators of crop pests in the Indian Union, the species described in the following pages were reared in the laboratory. There are very few records of parasites on thrips in the world and there is only one record from the Indian Union. In this paper two species of the genera *Dasy-scapus* and *Tetrastichus* have been described as new to science. Two new species of the genus *Anastatus* and a species of *Closterocerus* have also been described as new to science. Types have been deposited in the "National Pusa Collection", I.A.R.I., New Delhi.

EULOPHIDAE

Dasy-scapus thripsivorous, new species

Female :—Length 0.5 mm. to 0.6 mm. Head and thorax darkish brown; antennae, abdomen and legs light yellowish.

Head transverse, as wide as long, eyes strongly divergent below, vertex and frons smooth, frontoclypeal suture forming a distinct straight line between the compound eyes. Antennae inserted above the line joining the lower extremities of the eyes on two lateral protruberances which are separated by the triangular shaped labrum. Antennae eight segmented; scape long, thicker at the base and narrowed apically; pedicel more than half of the length of the scape; ring joint very narrow and not very distinct except in well prepared mounts; funicle joints a little longer than broad; club four jointed, more or less equal in length to that of scape, distinctly thicker than the pedicel, the joints subequal in length, the apical joint slightly longer than the rest and terminating in a prominent spine and beset with several long bristles.

Thorax smooth :—Mesonotum with distinct parapsidal furrows; scutellar base with one long spine laterad on either side. Wings hyaline, narrow at the base; marginal vein slightly longer than the submarginal; stigmal vein very short. Fringe at the apical margin more than twice as long as the maximum width of the wing.

Abdomen a little shorter than the length of the head and the thorax combined, sub petiolate, more or less ovate and pointed at the apex. Ovipositor sheath prominent but not protruding beyond the apex of the abdomen.

Male :—Length 0.5 to 0.6 mm. Scape abnormally swollen and dark brownish in colour. Apical one-third of the abdomen slightly black, otherwise essentially similar to the female.

Host :—A species of thrips feeding on the groundnut.

Locality :—Ratnagiri (Bombay State).

Collected :—M. Ramachandra Rao, February 1958.

Holotype :—One female on slide. **Allotype** :—One male on slide.

Dasyscapus thripsivorous, new species can be easily distinguished from *Dasyscapus parvipennis* (Gahan) by the following characters:

1. Pedicel not equal in length to the ring joint and the following two joints combined.

2. Club distinctly thicker than the pedicel.

3. Abdomen without any blackish or brownish spot on each side near the apex in the female.

Closterocerus agromyzae, new species

Female : Length 2 mm. to 2.1 mm. Head transverse, occiput minutely reticulate; vertex closely punctate reticulate with two bristles anterior to each lateral ocellus. Intero-cellular space twice the ocellular space. Face deeply excavate, antennae inserted in the face cavity slightly above an imaginary line joining the lower extremities of the eyes. Dorsad and laterad, the scrobes minutely punctate reticulate with greenish reflections. Antenna apparently eight jointed, highly flattened and leaf like with short spinose bristles and sensoria all over and with one ring joint hidden in the cavity of the pedicel. Scape narrow at the base, broadening distally; pedicel half as long as the scape; first three funicular segments more or less subequal in length but the third segment a little narrower than preceding; fourth segment very slightly shorter but distinctly narrower than the preceding; club unjointed, bulb-like at the base and tapering into a long terminal style apically.

Thorax :—Pronotum narrow and closely punctate reticulate with two lateral bristles on the pleurae. Mesonotum broad with symmetrically arranged reticulations on the scutum and scutellum; axillae widely separated. Mesopleura with one prominent stout bristle on either side and another small bristle on each tegula. Metanotum and propodeum minutely punctate with bronze reflections in the middle; propodeum without a median carina.

Abdomen :—Slightly longer than thorax, ovate; first tergite conspicuously longer than the rest. Valves of the ovipositor slightly extending beyond the tip of the abdomen.

Wings :—Smoky, marginal vein of forewing more than twice longer than the submarginal and bearing thirteen long bristles of equal size dorsally; post marginal vein very short, stigmal vein thick and spatulate with a chain of four cells rising in the middle. Three fuscous transverse bands, one at the apex of the forewing border, the second just below the stigmal vein, the third almost in the middle of the marginal vein. The fuscous bands are intervened by hyaline space.

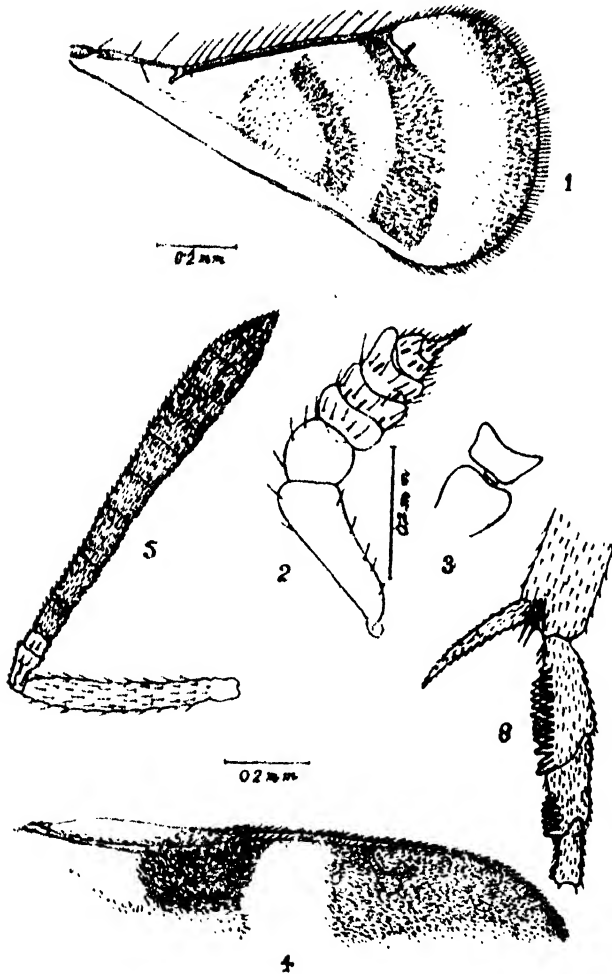
Legs :—Median tibiae and tarsi light yellowish and the rest fuscous; median tibia with a prominent stout spine as in the case of most of the encyrtids.

Holotype :—One female on slide, several paratypes on slides, some partly dissected.

Locality :—Delhi, collected B. R. Subba Rao, January 1957.

Host :—*Agromyza* species.

Closterocerus agromyzae, new species comes very near to that of *Closterocerus javanus* Perkins but differs from it in the size and length of the ring joint (pedicel is longer than the ring joint and the two following funicular joints combined in case of *C. javanus*). While *C. javanus* possess a distinct roundish smoky spot occupying the middle of the wing from costal to dorsal margin, *C. agromyzae* possess three concentric a species of the fuscous bands across the wing. This is the first record of a species of the genus from India.



TEXT-FIGURE 1.

1. Fore wing of *Closterocerus agromyzae*, new species ♀.
2. Antenna of *C. agromyzae*, new species ♀.
3. Pedicel, ring joint and funicle (highly magnified) ♀.
4. Fore wing of *Anastatus dentatus*, new species ♀.
5. Antenna of *A. dentatus*, new species ♀.
6. Middle tibial spur and tarsal segments of *A. dentatus*, new species ♀.

TETRASTICHIDAE

Tetrastichus rhipophorathripscidis, new species

Female :—Head, thorax and distal two-third of the abdomen dark brown; scape of antennae and legs light yellowish, rest of antennae dark yellow. Length 1.17 mm.

Head :—Face slightly longer than broad, vertex convex with transverse reticulations; interocellar (4) one and one-third longer than ocellular (3). Frons laterad and dorsad of the scrobe with distinct transverse reticulations. Antennae inserted above an imaginary line joining the lower orbits of the eyes. Scape cylindrical, a little more than two times the length of the pedicel; ring joint compound with the ramae fused; funicular joints almost equal in length except for the first which is slightly longer than the rest; club three jointed, distinctly wider than the funicular segments, slightly shorter than the scape. Club and the funicular segments beset with fine bristles. Mandible bidentate, margin of the ventral tooth minutely serrate, dorsal tooth with two distinct bristles.

Thorax :—Pronotum narrow and transverse. Scutum broad, infusate with three bristles in a row on either side laterad; scutellum with two bristles laterad on either side. Mesonotum with two small bristles one on each side just below the apical carina. Propodeum with median and paraspiracular carinae absent, with two bristles in a row laterad; spiracles contiguous with anterior margin, with a distinct long bristle adjacent to each on the apical margin.

Wings hyaline; submarginal vein of the forewing with two long dorsal bristles, slightly shorter than the marginal; marginal vein with nine distinct long bristles, apex of the hind wing acute, the posterior margin with a long fringe.

Abdomen not distinctly petiolate, slightly longer than the thorax, anterior two-third of the venter hyaline or light yellowish, proximal one-third of the dorsum light brown and the distal two-third dark brown or even black. Ovipositor sheath prominent and slightly projecting beyond the apex of the abdomen and bearing four long hairs.

Male :—Essentially similar to that of the female but for the densely hairy antennae.

Tetrastichus rhipophorathripscidis, new species runs very close to *Tetrastichus thripoponus* Waterston, but differs from it in the following :

1. Interocellar space only one and one-third longer than the ocellular space.
2. Antennae inserted above an imaginary line joining the lower extremities of the eyes.
3. Club distinctly wider than the rest of the antennal segments.
4. Mesonotum with three lateral bristles in a row on either side.

Holo and allotypes on a single slide. Paratypes dissected and mounted on slides.

Host :—*Rhipophorathrips cruentatus* Hood.

Collected :—Ramachandra Rao, June 1958, Delhi.

FAMILY EUPELMIDAE

Anastatus acherontiae, new species

Female :—Length 2 mm. to 2.1 mm.

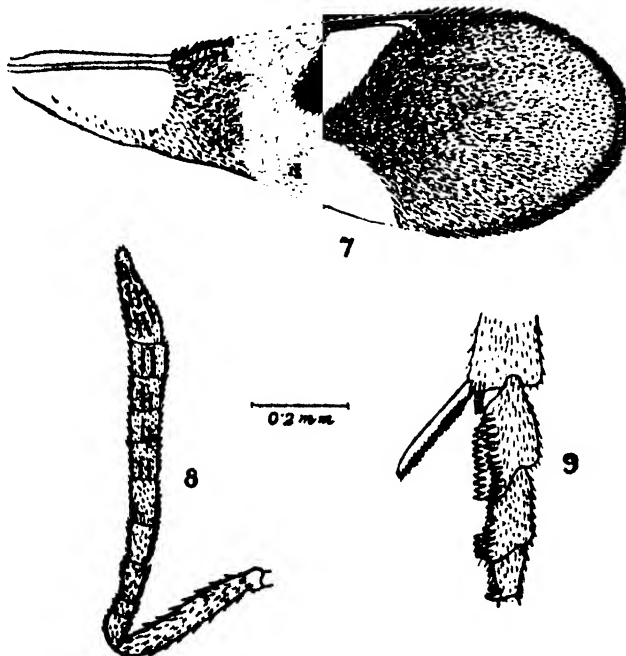
Head :—Transverse, more than twice broader than thick antero posteriorly. Vertex, face and the entire scrobe cavity rugulose with irregular and closely arranged

striations; scrobes not very deep and not reaching the anterior ocellus. Inter-ocellar space slightly more than twice the ocellocular. Malar space less than half as long as the eye. Antennae thirteen jointed, inserted just below an imaginary line joining lower extremities of the compound eyes. Scape extending to the vertex, slightly bent in the middle. Pedicel more than twice longer than the first funicular segment. Second, third and fourth segments more or less equal in length; sixth segment slightly shorter than the preceding segments; sixth, seventh and eighth joints subequal, wider than the preceding but shorter in length. Club three jointed, as long as the last three funicular segments combined.

Thorax :--Pronotum smooth and impunctate. Median lobe of the mesoscutum with distinct symmetrically arranged reticulations, the lateral lobes smooth and strongly shining. Scutellum and axillae sculptured exactly like the median lobe of the mesoscutum but for the more closely arranged reticulations. Mesopleura faintly lineolated and shining. Submarginal vein of the forewing as long as the marginal. Post marginal, more than twice longer than the stigmal vein.

Abdomen :--More or less equal in length to the thorax, broadening gradually from base to apex, where it is acuminate. First tergite with a hyaline band.

Colouration :--Head metallic bronze with greenish reflections, scape yellowish, pedicel and flagellum fuscous, mesoscutum light fuscous on the lateral lobes. Median lobe, scutellum and axillae metallic bronze with greenish iridescens. Propodeum infusate with greenish reflections. Pleurae and legs light fuscous brown. Wings dusky with two hyaline ovate patches adjoining the apical and inner margins situated in the central part of the wing and another hyaline patch at the wing base. Abdomen mostly black with the proximal one-fourth hyaline or light yellowish.



TEXT-FIGURE 2. .

7. Fore wing of *Anastatus acherontiae*, new species ♀.

8. Antenna of *A. acherontiae*, new species ♀.

9. Middle tibial spur and tarsal segments of *A. acherontiae*, new species ♀.

Male :—Not known.

Holotype :—One pinned specimen, described from six females.

Host :—*Acherontia styx* Westw.

Collected :—M. Ramachandra Rao, Delhi, April, 1959.

Anastatus acherontiae, new species differs from all the known oriental species in length, colouration and hyaline spots of the wing; the absence of hyaline band across the width of the wing.

Anastatus dentatus, new species

Female :—Length, 2 to 2.5 mm. Head transverse, more than twice wider than thick anteroposteriorly, vertex and face rugulose, ocelli arranged in an equilateral triangle, lateral ocelli very near the eye borders but not touching. Malar space nearly half as long as the compound eyes, sculpturing similar to that of the vertex and face, malar furrow distinct. Antennae inserted level with the lower extremities of the eyes, bearing short hairs all over. Scape elongate and slightly bent in the middle, about 0.52 mm. long, longer than pedicel, annulus and first three funicular segments together. Pedicel longer than broad, annulus short not broader than pedicel. First two funicular segments subequal, third slightly longer than the preceding; fourth as long as the first but slightly wider; fifth a little longer than the sixth; sixth and seventh subequal; club three jointed broad at the base and narrow at the apex, about one-third as long as funicle. Scrobes not reaching the anterior ocellus, deep at the base and shallow at the apex.

Thorax : One and half times longer than broad. Prothorax viewed from above triangular with a longitudinal hyaline strip in the middle. Scutum closely, honey comb like reticulated; scutellum and axillae with closely arranged reticulations; axillae widely separated; mesopleura finely lineolated; propoderum narrow in the middle, wider laterally. Wings with a hyaline band in the middle, the posterior margin slightly angulated near the lower end. Submarginal vein slightly longer than the marginal, post-marginal vein less than half as long as marginal; stigmal vein nearly half as long as post-marginal.

Legs : Tibial spur of the middle leg as long as the first tarsal segment. Middle tibia with four sharp small teeth at the apical angle. First tarsal segment of the middle tibia with fourteen short spines in a row. Second segment with six spines in a row; third and fourth segments with two spines each.

Abdomen :—As long as thorax, narrow at the base, broadening gradually and rounded at the apex. First and second segments with a transverse hyaline band and lateral darkish brown spots on the first tergite. Ovipositor slightly exerted.

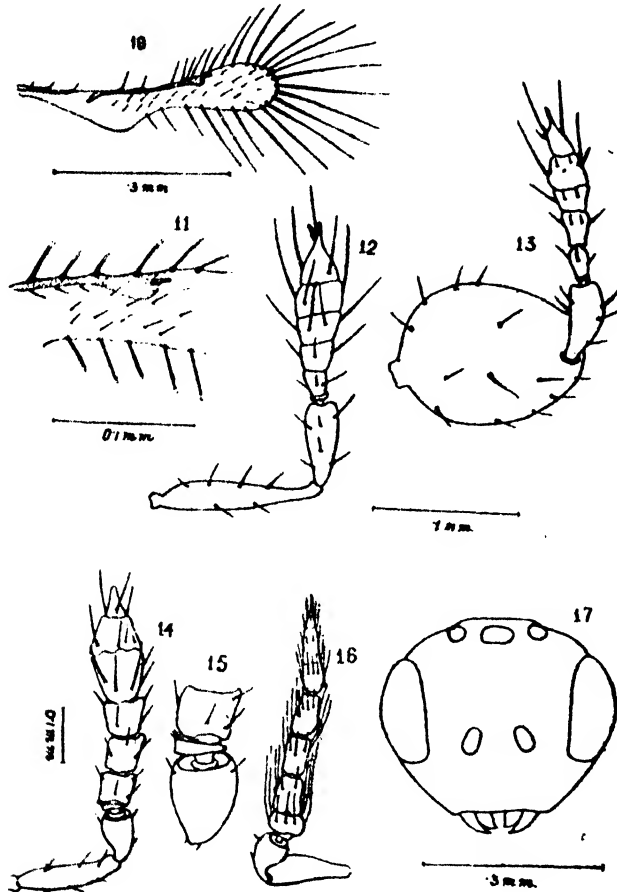
Colouration :—Head bluish green with purplish and aeneous reflections, scape of the antenna yellowish brown, rest of the antennal segments dark grey. Thorax darkish green with purplish reflections. Abdomen bluish green with a hyaline band at the base. Legs fuscous brown. Wings smoky with a transverse hyaline band in the middle and a hyaline patch at the base.

Male :—Half as long as female. Head, thorax and abdomen bluish green with purplish reflections; legs darkish blue with the exception of tarsal segments which are hyaline; antenna brownish black. Head, transverse, three times wider than long antero posteriorly. Vertex finely rugulose, post ocellar space twice longer than the ocellular. Face minutely punctate laterad the scrobes; scrobes deep and nearly reaching the anterior ocellus. Eyes grayish brown. Malar space as long as the compound eyes, malar furrow distinct. Scape short and thick, not reaching the anterior ocellus; pedicel very short and very much narrower than the scape; first funicular segment slightly bent in the middle and a little longer than the next

two segments which are subequal; fourth slightly longer than the fifth and the sixth which are again subequal; seventh distinctly smaller than the sixth and narrower. Club as long as the first funicular segment, joints indistinct, narrowed laterad towards apex.

Thorax.—Convex; prothorax narrow and transverse. Mesonotum with the parapsidal furrows distinct and complete; entire mesonotum minutely punctate and lineolate, as wide as the head. Propodeum narrow in the middle with a weak median carina.

Wings.—Hyaline; submarginal vein one and half times longer than the marginal vein, post-marginal less than half as long as marginal. stigmal vein more than half of the postmarginal.



TEXT-FIGURE 3.

10. Fore wing of *Dasyscapus thripsivorous*, new species ♀.
11. Fore wing highly magnified showing the marginal and stigmal veins of *D. thripsivorous*, new species ♀.
12. Antenna of *D. thripsivorous*, new species ♀.
13. Antenna of *D. thripsivorous*, new species ♂.
14. Antenna of *Tetrastichus rhipophorathripscidis*, new species ♀.
15. Antenna of *T. rhipophorathripscidis*, new species, highly magnified to show the fused segments.
16. Antenna of *T. rhipophorathripscidis*, new species ♂.
17. Head of *T. rhipophorathripscidis*, front view ♀.

Abdomen :—As long as the thorax, narrow at the base and broadest at the apex. Holo and allo type on slides, paratypes on card mounts.

Collected :—M. Ramachandra Rao, Delhi, 1959.

Host :—*Halis dentata* Fb.

Anastatus dentatus, new species differs from the nearest related species, *Anastatus kashmirensis* Mathur in the following. Overall length of the parasite, bluish green colour of the abdomen, ratio of the antennal segments. *A. dentatus* also differs from *A. colmani* Crawford in the length of the antennal segments: position and width of the hyaline band of the forewing.

KEY TO INDIAN SPECIES OF *Anastatus* ♀♀

1. Females with a hyaline band across the fore wing..... 2
Females with hyaline patches on the fore wing..... 6
2. Hyaline band curved or angulated.....
Hyaline band straight..... *Anastatus bangalorensis* Mani & Kurian
3. Pedicel nearly equal or slightly longer than the first funicle joint..... 4
Pedicel shorter than the first funicle joint..... 5
4. Hyaline band slightly curved; nearly as wide as the length of the stigmal vein below the marginal vein; abdomen bluish green
..... *Anastatus dentatus*, new species
Hyaline band slightly curved; nearly three times broader than the length of the stigmal vein; abdomen brown. . . . *Anastatus kashmirensis* Mathur
5. Hyaline band of fore wing strongly angulated, abdomen black
..... *Anastatus blattidarum* Ferriere
Hyaline band of fore wing slightly angulated; abdomen brown
..... *Anastatus colmani* Crawford
6. Fore wing with three hyaline patches; pedicel longer than the first funicle; abdomen dark brown..... *Anastatus amarus* (Subba Rao)
Fore wing with three hyaline patches; pedicel shorter than the first funicle; abdomen mostly black..... *Anastatus acherontiae*, new species

Mani (1938) has catalogued *Anastatus coimbatorensis* Girault. But its description has never been published.

ENCYRTIDAE

Dusmetia indica Burks, a synonym of *Dusmetia sanguani* Subba Rao.

Subba Rao (1957) described *Dusmetia sangprani*, a parasite of *Antonina graminis* (Mask.). Burks (1957) described *Dusmetia indica*. However, in his description Burks stated that the combination of characters (of this species) strongly suggest the genus *Dusmetia* Mercet 1921 without entirely agreeing with the description of it (t.c.). But he pointed out that the characters in agreement with *Dusmetia* Mercet were more than the differences, and hence there was no need to erect a genus of doubtful validity.

Dr. Burks informed one of the authors that there is no doubt about his *D. indica* being a synonym of *D. sanguani* according to rules of priority.

ACKNOWLEDGEMENT

The authors express their grateful thanks to Dr. Burks of the United States National Museum, who has very kindly agreed to the two species being synonymised.

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THE CRANIAL MUSCULATURE OF A HILL-STREAM CYPRINID FISH *GARRA MULLYA* (SYKES)

by SUBHASH CHANDRA SAXENA, Zoology Department, University of Delhi, Delhi
(communicated by M. L. Bhatia, F.N.I.)

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ABSTRACT

Cranial muscles of the hill-stream Cyprinid fish *Garra mullya* (Sykes) are grouped under the lateral, the dorsal and the ventral cranial muscles, the masticatory muscles and the branchial muscles.

Stress has been laid on the muscles associated with the adhesive apparatus and with the feeding mechanism of the fish.

The functional rôle of each muscle has been dealt with in detail.

The general plan of the musculature of *G. mullya* resembles that of other Cyprinids but the intermandibularis and the geniohyoideus muscles, which are responsible for the functioning of the disc, are highly modified. The intermandibularis muscle is infiltrated partly by fatty and tendinous tissues and is not fully developed.

INTRODUCTION

Ecological conditions in the hill-streams have brought about various interesting modifications in the morphology of torrential fishes. The chief modifications are those associated with the feeding mechanism of the fish. With a view to understanding these modifications, the study on the cranial muscles of a hill-stream Cyprinid fish *Garra mullya* (Sykes) was undertaken.

Various aspects of the cranial muscles of fishes have been studied by several workers. Among the earlier investigators, mention may be made of Vetter (1874, 1878); Allis (1897, 1903) on *Amia calva* and *Scomber*; Haempel (1908) on Cyprinoids. Takahasi (1925) gave a comparative account of the phylogenetic significance of each cranial muscle in Cypriniform fishes; Edwards (1926) and Eaton (1943) on the muscles responsible for the protraction of jaws in Catostomid and Cichlid fishes respectively. Recent papers on the cranial muscles associated only with the feeding mechanism are by Al-Hussaini (1949) on *Cyprinus carpio*, *Gobio gobio* and *Rutilus rutilus*, and Girgis (1952) on *Labeo horie*. More recently Nawar (1955) published a paper on the cranial muscles of a Siluroid fish *Clarius lazera*. From the above review it is seen that little attention has been paid to any of the Indian hill-stream fishes.

The material for investigation was collected in 1948 from the shallow rivulets of the Aravalli Range of Rajasthan, India. Fishes used for dissections, varying from 30 mm. to 60 mm. in standard length, were preserved in 70 per cent alcohol. The dissections were done under a binocular dissection microscope. Nomenclature of the muscles adopted, is that followed by previous workers, but certain new names assigned on the basis of function of the muscles, have been introduced. The names of the masticatory muscles and some of the lateral muscles of the head, are after Girgis (1952).

LATERAL CRANIAL MUSCLES (Figs. 1, 2, 3)

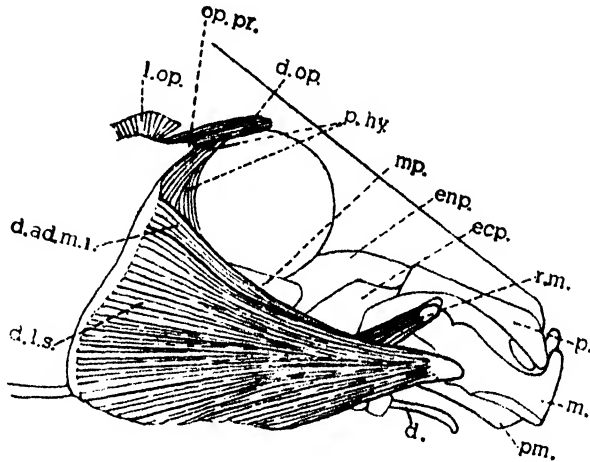
The lateral cranial muscles are divided into superficial and deep lateral muscles. The superficial muscles lie immediately underneath the skin and consist of protractor hyomandibularis, dilator operculi, levator operculi and depressor labii superioris.

The deep lateral muscles lie below the superficial muscles and consist of adductor operculi, retractor maxillaris and adductor hyomandibularis.

Protractor hyomandibularis (*p. hy.*). The muscle lies behind the orbit. Its origin on the sphenotic is below the dilator operculi. Just below the insertion of these muscle fibres, another set of muscle fibres of the same muscle originates and is attached on the hyomandibula above the origin of adductor mandibularis. The function of the protractor hyomandibularis is to elevate the hyomandibula forward and thus to enlarge the buccal cavity.

Dilator operculi (*d. op.*). The muscle lies dorsal to the protractor hyomandibularis. It has a narrow origin from the ventral surface of the frontal and is attached to the opercular process of the opercular bone. The contraction of this muscle dilates the opercular aperture and thus helps the water current to pass out.

Levator operculi (*l. op.*). It is a flat thin muscle that lies behind the dilator operculi. The muscle originates from the pterotic and is inserted on the inner side of the dorsal edge of operculum. The contraction of this muscle elevates the operculum and brings its marginal flap nearer to the cleithrum to close the opercular aperture.



TEXT-FIG. 1.

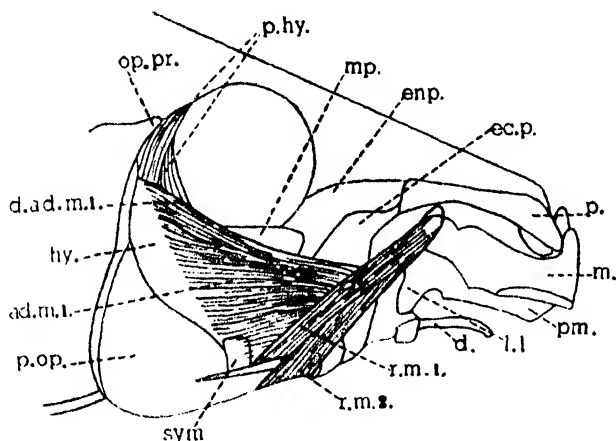
FIG. 1. *Garra mullya* (Sykes). Lateral cranial muscles (superficial). *d.*, dentary; *d.ad.m.I.*, dorsal fibres of adductor mandibularis I; *d.l.s.*, depressor labii superioris; *d.op.*, dilator operculi; *ecp.*, ectopterygoid; *enp.*, endopterygoid; *l.op.*, levator operculi; *m.*, maxilla; *mp.*, metapterygoid; *op.pr.*, opercular process; *p.*, palatine; *p.hy.*, protractor hyomandibularis; *pm.*, premaxilla; *r.m.*, retractor maxillaris.

Adductor operculi. The muscle lies below the levator operculi and internal to the dorsal edge of operculum. The deeper part of the muscle originates from the pterotic and the prootic bones, while the superficial part takes its origin from the inner side of pterotic articular facet of hyomandibula. The muscle runs ventrally backwards and is attached on the inner side of the dorsal edge of operculum. This muscle adducts the operculum.

Depressor labii superioris (*d.l.s.*). It is the largest and most superficial muscle of the head, lying immediately under the skin on the lateral sides. It originates on the posterior lateral marginal surface of the hyomandibula and the lateral surface of preoperculum, reaching upto its ventral edge. A few of the most dorsal fibres also originate only from the hyomandibula. All the muscle fibres converge into

a strong tendon which attaches on the lower edge of the maxilla in close contact with the anterior lip. Contraction of this muscle on both the sides depresses the anterior lip and thus it is partly responsible for the closing of the mouth. The posterior lip is non-protractile and takes no active part in its closure.

Retractor maxillaris (r. m.). Major part of the muscle lies under the depressor labii superioris except its anterior part, adhering to the maxilla which remains exposed. It takes origin by two heads—the retractor maxillaris 1 and retractor maxillaris 2, which originate from the different bones. Both the parts run together anteriorly but remain separate up to a considerable distance and then blend together into a common tendon to get attached on the lateral surface of the posterior part of maxilla, dorsal to the depressor labii superioris. The retractor maxillaris 1 and 2 originate from the quadrate and the ventral margin of the preoperculum respectively. At the origin, the dorsal fibres of retractor maxillaris 2 run over the ventral fibres of the retractor maxillaris 1, but become internal to it anteriorly, where they blend with each other. The contraction of this muscle on both the sides retracts the maxilla along with the premaxilla and serves in closing the mouth.



TEXT-FIG. 2.

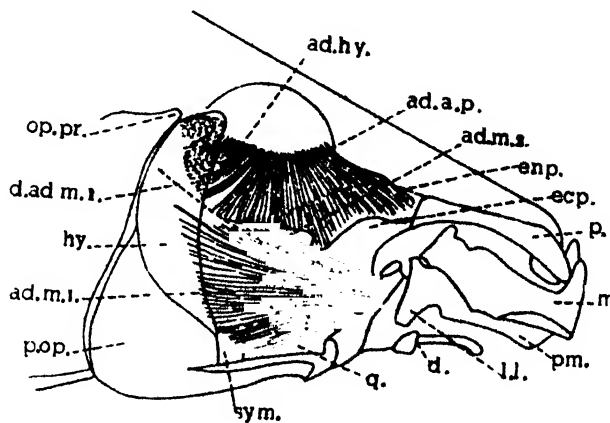
FIG. 2. *Garra mullya* (Sykes). Lateral cranial muscles (deep). *ad.m.1.*, adductor mandibularis 1; *d.ad.m.1.*, dorsal fibres of adductor mandibularis 1; *d.*, dentary; *ecp.*, ectopterygoid; *enp.*, endopterygoid; *hy.*, hyomandibula; *l.l.*, lateral limb of premaxilla; *m.*, maxilla; *mp.*, metapterygoid; *op.pr.*, opercular process; *p.*, palatine; *p.hy.*, protractor hyomandibularis; *pm.*, premaxilla; *p.op.*, preoperculum; *r.m.1.*, retractor maxillaris 1; *r.m.2.*, retractor maxillaris 2; *sym.*, symplectic.

Adductor mandibularis. The muscle lies immediately below the depressor labii superioris and consists of two parts, the adductor mandibularis 1 and 2 (*d.ad.m.1.*, *ad.m.1.*, *ad.m.2.*). The adductor mandibularis 1 is a broad flat muscle. A mark of bisection separates the dorsal fibres of the muscle for a short distance posteriorly. The muscle as a whole has a broad origin on the medial surface of the hyomandibula but a few ventral fibres have their origin on the symplectic. Adductor mandibularis 2 originates from the lateral surface of the metapterygoid and fuses with the inner fibres of the adductor mandibularis 1. The muscle fibres originating from the hyomandibula run forward, obliquely and ventrally, while those from the symplectic run dorsally forward. The broad insertion of the muscle is on the dentary and angular. The simultaneous contraction of both the side muscles, adducts the mandible in exactly the same way as stated by Girgis (1952) for *Labeo horie*.

DORSAL CRANIAL MUSCLES (Fig. 3.)

The dorsal muscles of the head comprise of the adductor hyomandibularis and the adductor arcus palatinus.

Adductor hyomandibularis (*ad. hy.*). The muscle originates from the prootic and the parasphenoid bones, runs laterally outward and gets attached to the inner surface of cranial part of hyomandibula. The muscle is continuous at origin and insertion but the muscle fibres which originate from the prootic are short and are attached to the proximal cranial end of hyomandibula. The rest of the muscle fibres originate from the parasphenoid and are longer. Contraction of this muscle adducts the hyomandibula with its associated bones resulting in the contraction of the pharyngeal cavity.



TEXT FIG 3

FIG 3 *Garra mullya* (Sykes) Deep lateral and dorsal cranial muscles. *ad.a.p.*, adductor arcus palatinus; *ad hy.*, adductor hyomandibularis; *ad m.1.*, adductor mandibularis 1; *ad.m.2*, adductor mandibularis 2; *d.*, dentary; *d.ad.m.1.*, dorsal fibres of adductor mandibularis 1, removed; *ecp*, ectopterygoid; *enp.*, endopterygoid; *hy.*, hyomandibula; *l.l.*, lateral limb of premaxilla; *m*, maxilla; *mp*, metapterygoid; *op.pr.*, opercular process; *p*, palatine; *pm*, premaxilla; *p op*, preoperculum; *q*, quadrate; *sym.*, symplectic.

Adductor arcus palatinus (*ad.a.p.*). It is a large uniformly flat muscle with a broad base that arises from the parasphenoid. The muscle fibres run forward, laterally outwards and slightly backwards. The fibres directed forward and laterally outward, adhere over the dorsal surface of the endopterygoid and nearly cover it underneath. The posteriormost fibres are attached to the dorsal edge of the metapterygoid. The principal action of the muscle is to narrow the buccal cavity, although the muscle fibres attached on the metapterygoid also depress the roof of pharynx.

VENTRAL CRANIAL MUSCLES (Figs. 4, 5.)

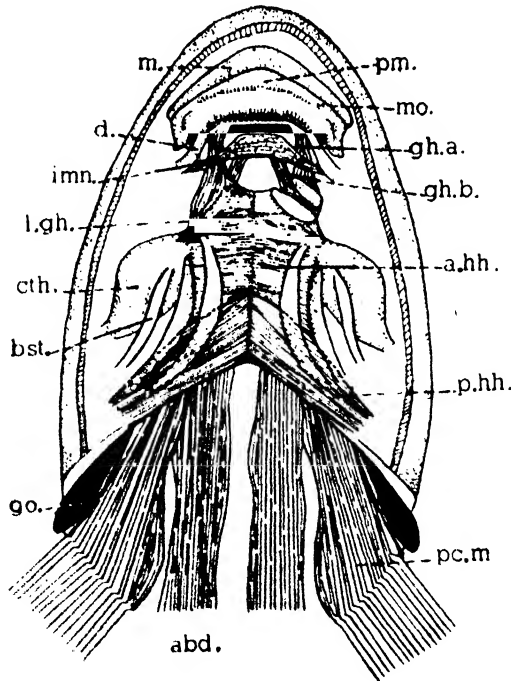
The ventral muscles of the head consist of intermandibularis, geniohyoideus, anterior and posterior hyohyoideus and sternohyoideus muscles. The presence of the disc on the ventral side of the mentum and the functional association of the geniohyoideus muscle with the disc have caused a modification in the muscles which is described later.

Intermandibularis (*i.mn.*). When the callous portion of the disc is peeled off superficially, both the intermandibularis and the geniohyoideus muscles get

exposed. Intermandibularis lies transverse to the median axis of the body, below the anterior margin of the callous portion, in between the mandibular arches. The muscle is partly mixed with the fatty and tendinous tissues and therefore presents an emaciated condition. It is ventral to the geniohyoideus *a* muscle and contraction of this muscle is partly responsible for creating vacuum inside the adhesive disc.

Geniohyoideus (*gh.a.*: *gh.b.*: *l.gh.*). The muscle originates from the outer surface of the ceratohyal and the head of the second branchiostegal ray. A few muscle fibers run forward dorsally to the intermandibularis, and are attached to the lower edge of the oral head of dentary. It is the geniohyoideus *a* muscle. But most of the muscle fibres diverge laterally to unite with those of the opposite side on the median axis of the body. To these lateral fibres is attached the posterior margin of the callous portion of the disc (where the callous part merges into the tuberculated border). A few posteriormost lateral fibres adhere on the anterior process of the urohyal. The lateral fibres from where they diverge, form a thick muscular belly. From the inner side of the muscular belly another muscle, geniohyoideus *b* originates on each side and is attached to the posterior surface of intermandibularis.

Contraction of the geniohyoideus *a* depresses the mandible, resulting in the opening of the mouth, while the simultaneous contraction of the lateral fibres of geniohyoideus, geniohyoideus *b* and the intermandibularis retracts the callous portion of the disc.



TEXT-FIG. 4.

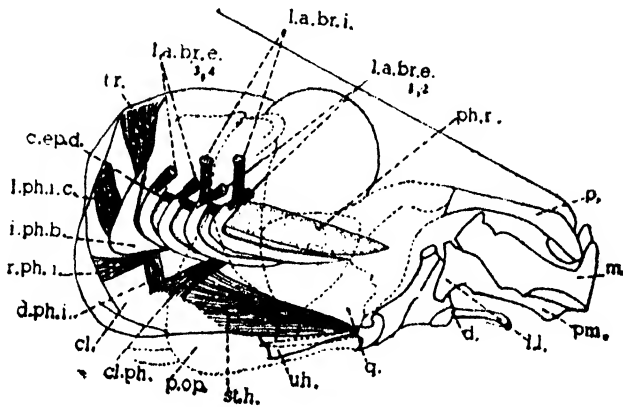
FIG. 4. *Garra mullya* (Sykes). Ventral cranial muscles. *abd.*, abdominal muscles; *a.hh.*, anterior hyohyoideus; *bst.*, branchiostegal rays; *cth.*, ceratohyal; *d.*, dentary; *gh.a.*, geniohyoideus *a*; *gh.b.*, geniohyoideus *b*; *g.o.*, gill opening; *imn.*, intermandibularis; *l.gh.*, lateral muscle fibres of geniohyoideus; *m.*, maxilla; *mo.*, mouth; *pc.m.*, pectoral muscle; *p.hh.*, posterior hyohyoideus; *pm.*, premaxilla.

Hyohyoideus. The muscle lies behind the geniohyoideus and some part under cover of the branchiostegal rays. Due to a transverse striation the muscle can be divided into anterior and posterior parts (*a.hh.*, *p.hh.*).

Anterior hyohyoideus muscle originates from the inner side of the ceratohyal and from the first and second branchiostegal rays. The muscle fibres run forward and obliquely inward to fuse with those of the opposite side over the urohyal on the median axis of the body. The anteriormost fibres of this muscle run below the posteriormost lateral fibres of the geniohyoideus muscle and are attached on the most anterior ventral surface of urohyal.

Posterior hyohyoideus muscle originates from the inner surface of the subopercle much more posteriorly than the anterior hyohyoideus. The muscle fibres run obliquely forward on the median axis in contact with the first and second branchiostegal rays.

The contraction of the anterior hyohyoideus narrows the buccal cavity. The action of the posterior hyohyoideus pulls the opercular bones and also partly constricts the pharynx.



TEXT-FIG. 5.

FIG. 5. *Garra mullya* (Sykes). Masticatory and branchial muscles. *c.ep.d.*, constrictor epibranchialis dorsalis; *cl.*, cleithrum; *cl.ph.*, cleithropharyngeus; *d.*, duetary; *d.ph.i.*, depressor pharyngeus interioris; *i.ph.b.*, inferior pharyngeal bones; *l.a.br.e.1,2,3,4.*, levator arcus branchialis externus; *l.a.br.i.*, levator arcus branchialis internus; *l.l.*, lateral limb of premaxilla; *l.ph.i.c.*, levator pharyngeus inferioris caudalis; *m.*, maxilla; *p.*, palatine; *ph.r.*, pharyngeal roof; *pm.*, premaxilla; *p.op.*, preoperculum; *q.*, quadrate; *r.ph.i.*, retractor pharyngeus inferioris; *st.h.*, sternohyoideus; *tr.*, trapezius; *uh.*, urohyal.

Sternohyoideus (*st. h.*). The muscle has a broad origin from the cranial surface of the cleithrum anterior to the cleithropharyngeus, and internal to the depressor pharyngeus inferioris muscles. The muscle runs forward and is attached to the dorsal surface of the urohyal, slightly fusing with the muscle of the opposite side. Insertion and action of this muscle seem to be identical in all the teleosts. The contraction of this muscle pulls the urohyal behind, thereby depressing the basibranchials and the basihyal. Its contraction through geniohyoideus muscle also causes slight protrusion of the upper jaw.

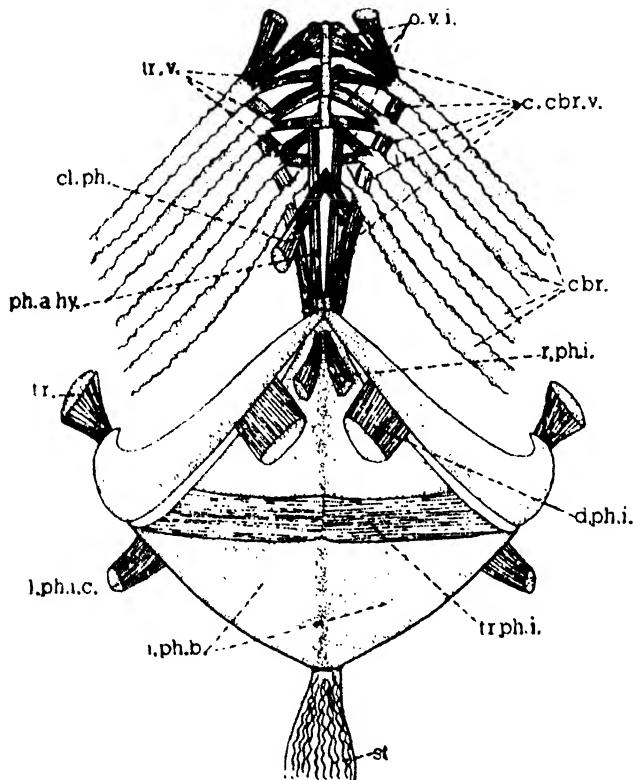
MASTICATORY MUSCLES (Figs 5, 6.)

This group of muscles is associated with the inferior pharyngeal bones which bear teeth for mastication. These masticatory muscles are trapezius, levator

pharyngeus inferioris caudalis, retractor pharyngeus inferioris, depressor pharyngeus inferioris and transverse pharyngeus inferioris.

Trapezius (tr.). It is a thick muscle having a broad origin on pterotic and exoccipital bones. The muscle on each side gradually tapers down and gets attached on the posterior surface of the dorsal process of the respective inferior pharyngeal bones so as to bring crowns of the pharyngeal teeth in contact with the ventral surface of the pharyngeal pad.

Levator pharyngeus inferioris caudalis (l.ph.i.c.). It is a pair of circular bundle of muscle fibres which arises from the posterior projection of the basioccipital (Pharyngeal process, Ramaswami, 1952). The muscles run ventrally forward and slightly lateral and are inserted on the lower parts of the posterior surface of the inferior pharyngeal bones. Contraction of this pair of muscle adducts and retracts the inferior pharyngeal bones in such a way that the crowns of the pharyngeal teeth rub against the surface of the pharyngeal pad.



TEXT-FIG. 6.

FIG. 6. *Garra mullya* (Sykes). Branchial and masticatory muscles (Ventral view). *cbr.*, ceratobranchial; *c.cbr.v.*, constrictor ceratobranchialis ventralis; *cl.ph.*, cleithropharyngeus; *d.ph.i.*, depressor pharyngeus inferioris; *i.ph.b.*, inferior pharyngeal bones; *l.ph.i.c.*, levator pharyngeus inferioris caudalis; *o.v.i.*, obliquus ventralis inferioris; *ph.a.hy.*, pharyngo-arcualis-hyoideus; *r.ph.i.*, retractor pharyngeus inferioris; *st.*, stomach; *tr.*, trapezius; *tr.ph.i.*, transverse pharyngeus inferioris; *tr.v.*, transverse ventralis.

Retractor pharyngeus inferioris (r.ph.i.). The muscle lies below the ventral surface of each inferior pharyngeal bone. On each side the muscle originates from the inner cranial surface of cleithrum slightly above its median angle, runs forward

internal to the depressor pharyngeus inferioris muscle, along the ventral surface of the inferior bone and is attached on its anterior ventral surface. Contraction of the muscles pulls the inferior pharyngeal bones back and slightly upward thereby rubbing the crowns of the pharyngeal teeth against the pharyngeal pad.

Depressor pharyngeus inferioris (d.ph.i.). It is a flat muscle which originates from the cleithrum externally to the origin of cleithropharyngeus and sternohyoideus. The inner fibres of the depressor pharyngeus inferioris are in close contact at origin with these muscles. This pair of muscles runs dorsally and obliquely backwards and is inserted on the ventro-lateral margin of the inferior pharyngeal bones externally to the retractor pharyngeus inferioris muscles. With the contraction of this pair of muscles, the inferior pharyngeal bones are pulled downwards thereby withdrawing the inferior pharyngeal bones from the surface of the pad.

Transverse pharyngeus inferioris (tr.ph.i.). This thin flat muscle stretches transversely on the ventral surface of the inferior pharyngeal bones. The muscle originates from the side edge of each bone, runs transversely, to blend with the muscle of the opposite side on the median suture of the two bones. The inferior pharyngeal bones are held together by this transverse band of muscles. When the muscles are lifted away, the bones are observed to separate out from each other.

BRANCHIAL MUSCLES (Figs. 5, 6)

The muscles associated with the branchial system of the fish are the cleithropharyngeus, constrictor ceratobranchialis ventralis, transverse ventralis, obliquus ventralis inferioris, pharyngo-arcualis-hyoideus, constrictor epibranchialis dorsalis, levator arcus branchialis externus and levator arcus branchialis internus.

Cleithropharyngeus (cl.ph.). The muscle originates from the cranial surface of the cleithrum, internal and slightly anterior to the depressor pharyngeus inferioris. A few anterior fibres of the muscle have their origin from the posterior part of the sternohyoideus muscle. The muscle runs dorsally forwards, fuses with the same muscle of the opposite side on the median axis and then is attached by a common tendon on the floor of the pharynx and partly also on the fourth ceratobranchial. The contraction of this muscle dilates the pharyngeal cavity.

Constrictor ceratobranchialis ventralis (c.cbr.v.). There are five constrictor ceratobranchialis ventralis muscles of which the first and the fifth are most prominent. The first muscle arises from the anterior end of the first ceratobranchial arch in contact with the obliquus ventralis inferioris muscle. The muscle runs forward obliquely and is attached on the inner surface of ceratohyal and hypohyal. The successive second, third and fourth muscles bridge between the first, second, third and fourth ceratobranchials respectively. The muscles become smaller in size posteriorly, the fourth being the smallest. The fifth muscle originates from the ventral process of the inferior pharyngeal bone and runs forward, dorsal to the cleithropharyngeus and gets attached on the ventral surface of the fourth ceratobranchial. Contraction of these muscles brings the ceratohyals, ceratobranchials and the inferior pharyngeal bones nearer to each other, narrowing the slits between them.

Transverse ventralis (tr.v.). It is a set of three of muscles which originate ventral to the obliquus inferioris from the ventral surfaces of the anterior parts of the first three ceratobranchials. The muscles run transversely and get attached on the respective hypobranchials but do not meet the muscles of the opposite side. Contraction of these muscles brings the ceratobranchials nearer together to the median axis of the fish body.

Obliquus ventralis inferioris (o.v.i.). The three obliquus ventralis inferioris muscles originate from the ventral surface of the anterior parts of the first three

ceratobranchials and in contact with the respective transverse ventralis muscles. The muscles run obliquely inward and get attached on the hypobranchials. The first muscle is the longest but the second and third are very small. Contraction of these muscles separates the ceratobranchials from each other.

Pharyngo-arcualis-hyoideus (ph.a.hy.). A pair of very thin muscle fibres originates from the antero-ventral process of the inferior pharyngeal bones. The muscles run forward parallel to each other. They are attached on the third hypobranch of each side. Contraction of the muscles raises the floor of the pharynx lying between the fourth ceratobranchial and the inferior pharyngeal bones.

Constrictor epibranchialis dorsalis (c.ep.d.). The muscle is represented by three parts which bridge over between the anterior portions of four epibranchials. The first two muscles originate from the prootic and the third and fourth muscles from the exoccipitals. The muscles bring the epibranchials closer to each other.

Levator arcus branchialis externus (l.a.br.e.). These are four serially arranged muscles attached on the anterior part of the dorsal surface of the epibranchials. The first two muscles originate from the prootic while the third and fourth arise from the exoccipitals. These muscles elevate the epibranchials.

Levator arcus branchialis internus (l.a.br.i.). The two levator arcus branchialis internus muscles originate from the prootic and are attached on the dorsal surface of the two pharyngobranchs.

FUNCTIONAL ROLE OF THE MUSCLES

The ventral position of the mouth of *Garra mullya* having a protractile anterior and non-protractile posterior lips, and jaws with horny covering and sharp cutting edges, is well adapted for scraping the algae from stones, rocks, etc. to which it remains attached. The jaws are protracted out of the mouth for this purpose by the contraction of muscles, geniohyoideus α and sternohyoideus. Contraction of these muscles depresses and slightly retracts the posterior jaw. This results in the protraction of the premaxillae, as their lateral limbs are attached with the dentary by ligaments. The premaxillae pivoting over their lateral limbs are obtruded beyond the oral head of the dentary, since it is attached dorsally to the rostrum by a considerably long inverted U-shaped rostral ligament. The anterior and the posterior jaws while they retract, scrape off the algae. The retraction of jaws is effected by the retractor maxillaris, which retract the premaxillae through the ligamentous attachment of maxillae with them and the adductor mandibularis by adducting the mandible. Thus the edges of the jaws come in contact with each other and food is drawn inside the buccal cavity. The contraction of the depressor labii superioris depresses the anterior lip, bringing it in contact with the posterior lip, and causing the closure of the mouth. In *Labeo horie* the food is procured by the highly protrusible lips which possess sharp horny cutting edges (Girgis, 1952). No such horny cutting edges are found on the lips of *G. mullya*, though the stratum corneum extends over the tubercles of the lips to form spines, and are similar to the horny protuberances described in *L. horie*. The lips of *G. mullya* perform an adhesive function, which has been dealt with in detail in a previous paper (Saxena, 1959).

As food and water enter the mouth, the buccal cavity dilates by the contraction of protractor hyomandibularis. But sooner it narrows as the contraction of anterior muscle fibres of adductor arcus palatinus depresses the roof and anterior hyohyoideus muscle elevates the floor of the buccal cavity. Thus the food is pushed back into pharynx. Now the levator arcus branchialis and the cleithropharyngeus contract thereby dilating the anterior pharyngeal cavity. Simultaneously, constrictor ceratobranchialis ventralis, transverse ventralis, and constrictor epibranchialis

dorsalis contract to bring the branchial arches close together and form a sieve for the filtration of water. The contraction of single cleithropharyngeus on each side can depress the ceratobranchials as they are held together by the ventral branchial muscles. With the opening of the mouth, water again gushes inside and the food is pushed still backwards as the pharyngeal floor is raised by the contraction of posterior hyohyoideus and pharyngo-arcualis-hyoideus. The contraction of the muscle fibres of the adductor arcus palatinus attached on the metapterygoid, and the adductor hyomandibularis depress the roof, which exerts pressure on the surface of the water contained in the pharyngeal cavity, to pass out the water through the gill-arches. The slits in between the gill-arches get widened by the contraction of the obliquus ventralis inferioris. As food is pushed to the back portion of pharynx, the depressor pharyngeus inferioris muscles depress the inferior pharyngeal bones and give access to food to enter the posterior part of the pharynx for mastication. The inferior pharyngeal bones now begin mastication. Trapezius muscle contracts to bring the crowns of the teeth in contact with the pharyngeal pad while the grinding is done by the contraction of levator pharyngeus inferioris caudalis and the retractor pharyngeus inferioris. The inferior pharyngeal bones act together as they are held by transverse pharyngeus inferioris muscle. When mastication is completed, the food passes inside the stomach by dilating the oesophageal opening.

The geniohyoideus muscle performs two-fold functions. The contraction of geniohyoideus *a* depresses the mandible thereby resulting in the protrusion of the upper jaw. Another important function is performed by the lateral muscles of geniohyoideus and the geniohyoideus *b* muscles in the adhesive mechanism of the disc. When the fish adheres to the substratum, the lateral fibres of the geniohyoideus and the geniohyoideus *b* along with the intermandibularis contract and create a vacuum inside the callous portion of the disc, thereby enabling fish to adhere through the disc suctionally.

DISCUSSION

The general plan of musculature of *Garra mullya* is similar to that of Cyprinids, described by Takahasi (1925). But the presence of a suctional disc on the ventral side of the mentum and shifting of the mouth to a more ventral position have resulted in the modification of the muscles associated with them.

The protractor hyomandibularis muscle of *G. mullya* is synonymous with levator arcus palatinus of other fishes studied by previous workers. The name attributed by Gregory (1933), justifies its function and has therefore been adopted.

The depressor labii superioris and retractor maxillaris correspond to Takahasi's (1925) A1 α and A1 β maxillaris parts of adductor mandibulae in *Cyprinus carpio* and *Pseudogobio esocinus*. These muscles have been described as united at origin in *Cyprinus* and totally separate in *Pseudogobio*. The retractor maxillaris or A1 α muscle is not described in *Labeo horie* by Girgis (1952).

All the Cyprinid fishes studied by Takahasi show the fusion of maxillaris muscle with mandibularis muscle either internally or at their origin. The depressor labii superioris and retractor maxillaris in *G. mullya* do not fuse with the adductor mandibularis or with each other. A comparative study by the author on the cranial myology of *Crossocheilus*, an allied genus of *Garra*, has revealed that the retractor maxillaris, though originating from the same bones as in *Garra*, is fused internally at the insertion with the overlying depressor labii superioris. Thus, it can be inferred that the depressor labii superioris and the retractor maxillaris also correspond to the single maxillaris muscle of *Opsariichthys*.

The muscle, levator labii inferioris, described by Girgis in *Labeo horie* is not present in *Garra*. However, due to the presence of a non-protractile posterior lip,

it can be presumed that the dorsal fibres of the adductor mandibularis 1, probably represent the atrophied condition of the levator labii inferioris.

The dorsal cranial muscles have been grouped under a single name "Adductor arcus palatinus," by many previous authors and under "Adductor hyomandibularis" by Gregory (1933). But in *Garra*, the muscle has been divided into two separate parts. The muscle attached to the hyomandibula is named as adductor hyomandibularis, while the rest of the anterior part of the muscle as adductor arcus palatinus. The same names have been used here owing to the separation of the hyomandibular part from the rest of the muscle and partly due to the difference in their functions.

Intermandibularis muscle has been reported poorly developed in Cyprinids (Takahasi, 1925) and totally absent in *Labeo horie* (Girgis, 1952). This muscle though in a degenerated condition, in *Garra* is fairly conspicuous due to its special mode of function in association with the geniohyoideus muscle.

The mode of operation of the muscles, geniohyoideus and the intermandibularis shows that the lower jaw is independent of the disc in movement, while the fish is attached. Geniohyoideus *a* muscle is only responsible for opening the mouth by depressing the mandible, while the simultaneous action of geniohyoideus *b*, the lateral fibres of the geniohyoideus and the intermandibularis creates vacuum in the disc and is thus independent of geniohyoideus *a* muscle. Thus it is evident that the adhesive apparatus of the fish does not affect the movements of the lower jaw. The Cyprinids which do not bear any adhesive apparatus on the ventral side of the mentum, usually lack the intermandibularis and also do not show any modification of the geniohyoideus muscle.

The present study of these muscles associated with the disc confirms the previous assumptions beyond doubt that the disc of *Garra* functions most efficiently as a vacuumatic sucker. When the fish adheres, it applies the callous portion of the disc to the substratum and contracts the intermandibularis, geniohyoideus *b* and the lateral fibres of the geniohyoideus. The contraction of these muscles retracts the callous portion of the disc from the substratum, thereby creating a vacuum. As stated in the previous paper (Saxena, 1959), the vacuum in the disc is maintained by the callous portion and not by the tuberculated border, as was thought by the previous workers.

The position, origin and insertion of hyohyoideus muscle in *Garra mullya* is similar to the hyohyoideus of *Cyprinus carpio* and *Amia calca*, studied by Takahasi (1925) and Allis (1897), respectively. The same muscle in *Labeo horie* has been described by Girgis (1952) as constrictor pharyngeus.

Takahasi (1925) described the presence of two trapezius muscles, (trapezius superficialis and trapezius profundus), in the Cyprinids he studied. But in *Garra* there is only one muscle corresponding to trapezius profundus. Taking into consideration the fact that the inferior pharyngeal bones of Cyprinids are the modified fifth branchial arch of other fishes (Goodrich, 1930, p. 441), it becomes evident that the various muscles associated with the inferior pharyngeal bones correspond to the muscles associated with the fifth branchial arches of those fishes. Therefore, Takahasi's statement in regarding the Vetter's fifth levator arcus branchialis externus becomes self-explanatory.

The levator pharyngeus inferioris caudalis muscle of *Garra* corresponds to Takahasi's superior and inferior parts of the retractor arcus branchialis dorsalis in *Cyprinus carpio*. The single pair of levator pharyngeus inferioris caudalis probably represents the fused state of the superior and inferior parts of retractor arcus branchialis which is concluded by his following statement :—

"It arises from the lateral surface of the cranium (masticating process of the basioccipital), runs forward, ventrad and outward, and is inserted on the dorsal

part of the fifth ceratobranchial ventral to the insertion of trapezius profundus." Further, regarding the inferior part of the muscle he states, "It is a rather stout muscle placed just ventral to the trapezius profundus and dorsal to the fifth transverse ventralis in its insertion, and external to the superior in its caput. Arising from the cranium (external surface of the masticating process of the basioccipital) the muscle runs forward, outward, and ventrad, and is inserted on the hind surface of the dorsal or posterior portion of the fifth ceratobranchial."

Takahasi groups a few other muscles associated with the fifth branchial arch. He identifies this set of muscles as pharyngo clavicularis internus and pharyngo clavicularis externus. Further, he describes the pharyngo clavicularis internus as anterior and posterior. From his description of the origin and insertion of these muscles in *Opsariichthys uncirostris*, it becomes evident that the posterior pharyngo clavicularis internus corresponds to the retractor pharyngeus inferioris of *G. mullya* and *L. horii* while the anterior pharyngo clavicularis corresponds to the cleithropharyngeus of these fishes. The pharyngo clavicularis externus of *Opsariichthys* is the depressor pharyngeus inferioris of *Garra* and *Labeo*.

Keeping in mind that the inferior pharyngeal bones of carps are homologous to the fifth branchial arch of other fishes, the homology between the transverse pharyngeus inferioris of *Garra* and *Labeo* with the fifth transverse ventralis muscle of the Cyprinoids dealt by Takahasi needs no explanation.

The first four ceratobranchialis ventralis muscles of *Garra* correspond to the four obliquus ventralis superioris muscles of the Cyprinids studied by Takahasi, while the fifth constrictor ceratobranchialis ventralis is the fourth transverse ventralis of *Opsariichthys uncirostris*, as is evident by the following description:

"The fourth ventralis originates from the ventral side of the anterior portion of the fourth ceratobranchial, and thus runs backward to its insertion on the external side of the anterior (ventral) portion of the fifth ceratobranchial of the same side."

Thus the only difference is the reverse origin and insertion of the muscle. But the action of the muscle in *Opsariichthys uncirostris* would be similar in this position to the action of the fifth constrictor ceratobranchialis of *Garra*.

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MODIFIED ROMANOWSKY STAINING OF THE SPINAL CORD AND THE CEREBELLUM

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(Communicated by N. M. Basu, F.N.I.)

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ABSTRACT

A new method has been developed for the differential staining of gray and white matter in spinal cord and cerebellum. The method essentially consists in, fixing the tissue in a modified Carnoy (acetone- 60; chloroform 30; acetic acid- 10) fixative. It is dehydrated in acetone and sections are prepared in the usual way. The sections are then stained with a mixture of Leishman and Giemsa (5:1) and afterwards treated with a suitable buffer. It is washed in water, dehydrated in acetone and mounted in balsam. It has been observed that at pH 4.8 there is a differentiation of gray and white matter in cerebellum, but not in spinal cord; the latter gives a differentiation at pH 6.5. It is possible that the isoelectric point of proteins of gray and white matter in spinal cord and cerebellum is different.

Romanowsky stain along with buffer has previously been used in tissue sections (Gatenby and Beams, 1950; Gude, Upton and Odell, 1955). In the present investigation a method has been developed for the differential staining of gray and white matter in spinal cord and cerebellum, using a mixture of Leishman and Giemsa stain and subsequent buffer treatment.

METHOD

1. Fix in a modified Carnoy fixative consisting of
Acetone — 60 ml
Chloroform — 30 ml
Acetic acid glacial — 10 ml
for 2-3 hours.
2. Dehydrate in 2-3 changes of absolute acetone.
3. Embed the tissue in paraffin and prepare 8 μ sections in the usual way.
4. Treat paraffin sections with xylol, acetone, acetone water (1:1) and hydrate to distilled water.
5. Stain with a mixture of Leishman and Giemsa (5:1) for 15-20 seconds.
6. Dilute the stain on the slide with an equal quantity of a buffer of suitable pH (depending on the nature of the tissue to be stained) and keep for 1 minute.
7. Throw off the stain and differentiate with acetone water till with the naked eye colour changes are noted.
8. Dehydrate rapidly with acetone.
9. Clear in xylol and mount in Canada Balsam.

Results : Spinal Cord

Gray matter	—	dark red
White matter	—	orange
Cellular matter	—	blue

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Cerebellum

Gray matter - molecular layer, bright pink; granular layer, blue dots due to stained nuclei, intercellular tissue pink.

White matter - light orange.

Purkinje cells - cytoplasm light blue, nucleus dark blue.

DISCUSSION

Effect of fixation and dehydration :

Spinal cord and cerebellum were fixed in Zenker, Bouin's, Formol and Carnoy. They were dehydrated in ethyl alcohol, and paraffin sections were stained by the method described. No differentiation between gray and white matter could be observed. Tissues fixed in Carnoy and dehydrated in acetone gave slight differentiation. For this reason tissues were subsequently fixed in a modified Carnoy in which the ethyl alcohol was replaced by acetone. It has been observed that only after fixation in this modified Carnoy and dehydration of both the tissue and the section with acetone, the gray and white matter could be well differentiated.

Effect of hydrogen ion concentration :

The hydrogen ion concentration of the diluting fluid after staining was found to be the determining factor in differentiating spinal cord and cerebellum. At pH 4.8 the gray and white matter of cerebellum could be very well differentiated in shades of red and blue, but the section of spinal cord turned all red; whereas at pH 6.5 both the gray and white matter of cerebellum turned blue, but spinal cord gave a differentiation. Phosphate and acetate buffer at M/5 and M/20 concentration were used and no difference could be observed either by changing the salts or the molarity of the buffer. It is possible that the isoelectric points of proteins of gray and white matter in spinal cord and cerebellum are different.

ACKNOWLEDGEMENTS

Grateful thanks are due to W.H.O. for the award of a fellowship and to Professor P. B. Sen, Professor of Physiology, for his valuable suggestions and encouragement.

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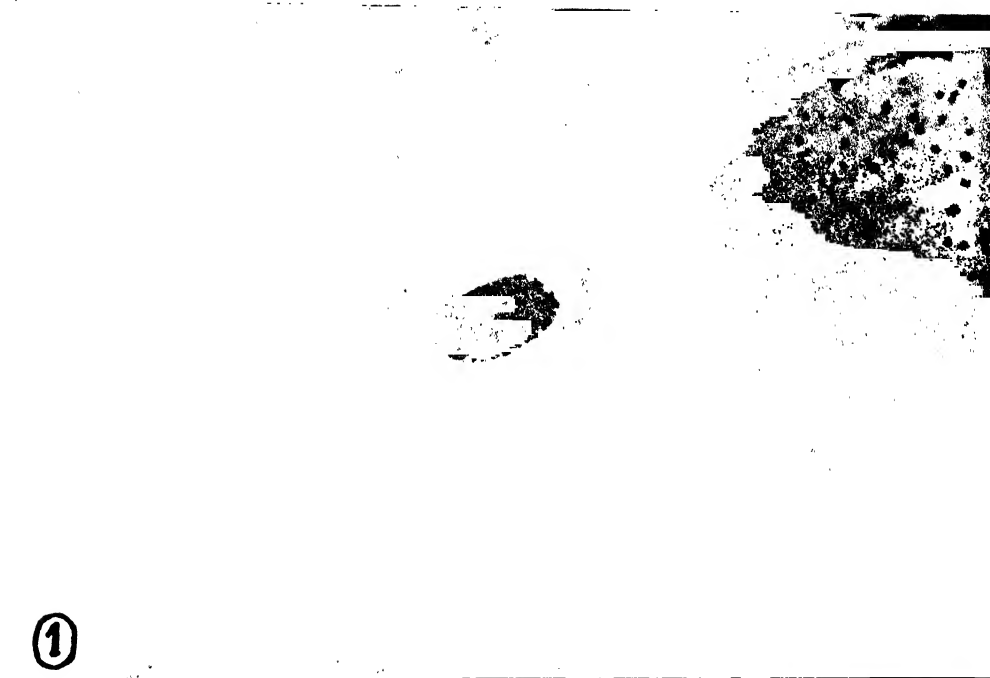


FIG. 1. Spinal cord of rat. Stained with Leishman-Giemsa and treated with M/20 phosphate buffer of pH 6.5. 60X
 FIG. 2. Cerebellum of rat. Stained with Leishman-Giemsa and treated with M/20 phosphate buffer of pH 4.8. 60X

ECOLOGY OF *ECLIPTA ALBA* HASSK.*

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Varanasi 5

(Communicated by R. Misra, F.N.I.)

(Received April 7, 1960)

ABSTRACT

Eclipta alba Hassk. is an annual plant thriving throughout the year. However, the incidence of the species is higher at the end of the rainy season and in the winter. The plant has a wide range of tolerance as regards moisture in the soil and its texture. It prefers a clayey soil with abundant moisture. This species can be conveniently classed under the less exacting group of calcicoles. The seed output for the plant is low in non calcareous soils and increases with the increase in lime. In highly calcareous soils the seed output value tends to fall down. The seeds give a germination of 62 per cent in diffused day light. In continuous light and after pretreatment of seeds with mud they give higher germination percentage. The capacity of seeds to withstand water-logged conditions of the soil enables the species to grow as a member of the drying pond bed flora. The reproductive capacity value for *E. alba* works out to 564. The stomatal frequency of the leaves is more in the open than in shade. The osmotic pressure values for this species are typical of a marshy plant.

INTRODUCTION

For an understanding of the biological and ecological equipment of a species in relation to environmental factors, its ecological life history has to be pursued. Importance of such life history studies has been stressed by Pelton (1951, 1953), Tansley (1946) and Whitehead (1957). Pelton (1951, 1953), Salisbury (1928), Anonymous (1941) and Anonymous (1958) have given elaborate schemes of study of ecological life cycle of plants.

Salisbury (1928) initiated the 'Biological Flora of British Isles' which was adopted by the British Ecological Society as a co-operative venture in 1951. Since then, ecological accounts of a number of species have appeared under this head in the subsequent issues of the *Journal of Ecology*. Besides, a good amount of detailed autecological studies have been done in Britain. Of these, the works of Mukerji (1936) on *Mercurialis perennis*, Conway (1936-1938) on *Cladium mariscus*, Blackman and Rutter (1946, 1947, 1948, 1949) on *Scilla non-scripta* and McVean (1955a, b, 1956a, b, c, d) on *Alnus glutinosa* are a few worth mentioning.

In India, ecological studies of herbaceous species have been initiated with the work of Misra and Siva Rao (1948). Misra and his students have given much attention to this type of study (Bakshi, 1952; Pandeya, 1953; Mall, 1956, 1957; Ramakrishnan, 1958; Misra and Ramakrishnan, 1959, etc.). The ecology of *Eclipta alba* as presented in this paper is further addition to the series of investigations.

Synonyms:

Eclipta erecta Linn., *Eclipta prostrata* Linn.

HABIT AND HABITAT

Eclipta alba Hassk. is an annual, erect or prostrate herb, often rooting at the nodes. The species grows at Varanasi in moist to water-logged localities such

* Part of a thesis submitted to the Banaras Hindu University for the award of a Ph.D. degree.

as along the margins of puddles, ponds and drainage channels. The plant is not infrequently met with in drier areas also. The species is a conspicuous member of the flora of drying ponds.

MORPHOLOGY

Root: The root system consists of finely branched thin roots penetrating to a depth of about 20 to 30 cm. Rootlets also arise from stem nodes wherever they are in contact with the substratum.

Stem: -Strigose with appressed white hairs rising from a thickened base.

Leaves: -Sessile, 2.5 to 10.0 cm long, but very variable, linear or oblong-lanceolate, subentire or toothed, narrowed at both ends.

Inflorescence: -Head subglobose 0.4 to 0.9 cm. diameter, solitary or two together on axillary short or long and slender peduncles. Heads heterogamous.

Involucral bracts: -About 8, obtuse or acute, strigose outside, about equalling or exceeding the flowers.

Ray flowers: Tubular, the corollas often 4-toothed. Pappus 0, except occasionally very minute teeth on the top of the achene.

Fruit: -Achenes cuneate compressed and with narrow wing, covered with warty excrescences.

GEOGRAPHICAL DISTRIBUTION

E. alba occurs throughout India and Ceylon ascending up to 6,000 ft. in the Himalayas and other mountains (Duthie 1905-1915). Distribution of the plant is pan-tropical.*

SIZE AND WEIGHT OF THE SEED

The seeds are slightly flattened with one end pointed and the other blunt. The length of the seed varies from 2.000 to 2.597 mm. and the breadth varies from 0.701 to 0.987 mm. The shape index (length/breadth ratio) varies from 2.03 to 3.70. The average weight of the seed is 0.25 mg.

ENVIRONMENTAL FACTORS

Climatic:

The climate of Varanasi is typical of the Upper Gangetic plains. The year is divisible into three seasons:

1. Rainy season - from last week of June to October.
2. Winter season - from November to February.
3. Summer season - from March to 3rd week of June.

E. alba grows throughout the year and is able to withstand low temperatures of the winter months as well as hot summer.

EDAPHIC FACTORS

The soil analysis data for collections from various localities are set in Table I. The analytical methods adopted are briefly indicated.

* This information was obtained from the Director, Royal Botanic Gardens, Kew, Richmond, Surrey.

TABLE I
Soil analysis data for incidence of *E. alba*

Locality	Moisture content (%)	pH	(Carbonate content (%))	Exchangeable calcium (m.e. %)	Nitrate content (mg./100g. of soil)	Organic matter (%)	Remarks
Samath	15.8	8.3	1.193	18.6	2.25	1.60	Margin of pond, open, clayey loam.
Rajghat	12.5	7.7	0.520	20.8	2.25	5.40	River margin, partly shaded, loam.
Akhari	26.8	8.0	0.158	8.0	2.75	4.00	Margin of pond, open, clayey soil.
University area	29.6	7.1	0.096	3.4	3.00	3.50	Water-logged, partly shaded, clayey soil.
Ramnagar	20.4	7.5	1.510	27.4	2.00	1.84	Along drainage channel, partly shaded, clayey soil.
Ganges bank	16.2	8.5	5.122	30.2	3.50	2.14	River margin, open, clayey soil.
Latifshaw	11.2	8.0	1.280	19.4	2.70	2.22	Open, drier locality, loamy soil, drying pond.

Hydrogen ion concentration was electrometrically determined. Carbonate content was determined by Hutchinson and MacLennan's method (as outlined by Piper, 1944). Exchangeable calcium was estimated by leaching the soil with N/2 solution of acetic acid and following the method described by Wright (1939) for calcareous soils. Nitrate in the soil was estimated colorimetrically following the method outlined by Snell and Snell (1949), using a Klett-Summerson's photo electric colorimeter. The nitrate was expressed as mg. of nitrate nitrogen per 100 g. of soil. Organic matter was determined by Robinson's method (as quoted by Wright, 1939).

PERFORMANCE OF THE PLANT IN VARIOUS LOCALITIES

The habit of the plant is very variable. It may be entirely erect or partly erect with some prostrate branches or wholly prostrate. The size of the leaves is also extremely variable. The average seed output of the plant is indicative of its success in different localities (Table II).

TABLE II
Average seed output of E. alba in various localities

Locality	Height of plant (cm.)	Average seed output	Remarks
Sarnath	7.0	774	Decumbent
Rajghat	10.0	818	do
Akhari	8.0	524	do
University area	6.8	342	Erect
Ramnagar	37.0	1514	Decumbent, rooting at nodes
Ganges bank		1597	Prostrate
Latifshaw	8.0	796	Erect

No definite correlation could be made out between the seed output of the plant and any of the factors like moisture content, pH, nitrate content and organic matter of the soil. However, definite correlation could be established between the average seed output and carbonate content and exchangeable calcium in the soil as is evident from a comparison of Tables I and II. The average seed output of the plant increases with an increase in exchangeable calcium and carbonate content of the soil.

ASSOCIATES

The important associates of *E. alba* from the various localities studied are given in Table III.

TABLE III

Associates of E. alba in various localities

Species	Localities*						
	1	2	3	4	5	6	7
<i>Eclipta alba</i>	o.f	f	o.f	f	a	o.f	f
<i>Alternanthera sessilis</i>	o.f			f	l.a		o.f
<i>Argemone mexicana</i>	r						
<i>Cassia tora</i>					f		
<i>Chrozophora rotterli</i>						o.f	
<i>Coldenia procumbens</i>			l.a				o.f
<i>Commelina benghalensis</i>				o.f	f		
<i>Crotalaria medicagenia</i>		o.f					
<i>Croton sparsiflorus</i>		o.f					
<i>Cynodon dactylon</i>		o.f					
<i>Cyperus</i> sp.	o.f		r	o.f			
<i>Digitaria sanguinalis</i>		o.f	r				
<i>Echinochloa colonum</i>	f			a	f		
<i>Eleusine indica</i>				f			
<i>Euphorbia hirta</i>			r				
<i>E. thymifolia</i>		r	o.f				
<i>Gnaphelium indicum</i>						o.f	f
<i>G. luteo-album</i>						f	f
<i>Lippia nodiflora</i>	a		l.a			o.f	f
<i>Marsilea</i> sp.				f			
<i>Mollugo hirta</i>	f						o.f
<i>Paspalidium flavidum</i>			f				
<i>Paspalum scrobiculatum</i>	f						
<i>Phyllanthus niruri</i>		r			o.f		
<i>Polygala chinensis</i>			r				
<i>Polygonum plebejum</i>			f				a
<i>Potentilla supina</i>						o.f	
<i>Setaria glauca</i>				r			
<i>Sphaeranthus indicus</i>						o.f	o.f
<i>Sporobolus diander</i>			o.f				
<i>Trianthema monogyna</i>					o.f		

*Localities: 1. Sarnath, 2. Rajghat, 3. Akhari, 4. University area, 5. Ramnagar, 6. Ganges bank, 7. Latifshaw.

a = abundant; l.a = locally abundant; f = frequent; o.f = occasionally found; r = rare absent.

CULTURE EXPERIMENTS

Experiment No. 1:- It is seen from the soil analysis data given in Table II, that *E. alba* occupies different textural grades of soil and also can tolerate a wide range of moisture levels.

To investigate into these factors and to ascertain how far the performance of the plant is affected by them, culture experiments were undertaken. Three sets, of three culture pots each, were prepared—(1) with clayey soil, (2) with clayey loam and (3) with sandy loam. In each of these sets one pot was kept waterlogged for about 15 minutes in the morning and moderately watered in the evening daily, another moderately watered twice a day and the third one moderately watered once a day in the morning. Two plants of *E. alba* were allowed to grow in each pot. The results are shown in Table IV (Pl. X, Figs. 1, 2 and 3).

TABLE IV
Effect of texture of soils and mode of watering on the performance of E. alba

Observations	Set 1 (Clayey soil)				Set 2 (Clayey loam)				Set 3 (Sandy loam)			
	Water-logged once a day	2 times moderately watered	1 time moderately watered	Water-logged once a day	2 times moderately watered	1 time moderately watered	Water-logged once a day	2 times moderately watered	Water-logged once a day	2 times moderately watered	1 time moderately watered	1 time moderately watered
Height of plant (cm.)	34.0	33.0	32.5	25.5	50.0	28.5	23.5	26.5	30.0			
No. of heads/plant	55	48	44	24	24	22	19	13	14			
Av. No. of seeds/head	45	40	42	47	36	37	40	47	41			
Seed output	2475	1920	1848	888	864	814	760	611	574			
Fresh wt. of shoot (g.)	18.54	17.64	17.60	8.80	8.82	8.40	7.81	8.20	8.15			
Dry wt. of shoot (g.)	13.66	13.22	10.46	3.10	6.62	5.80	3.00	5.64	4.98			

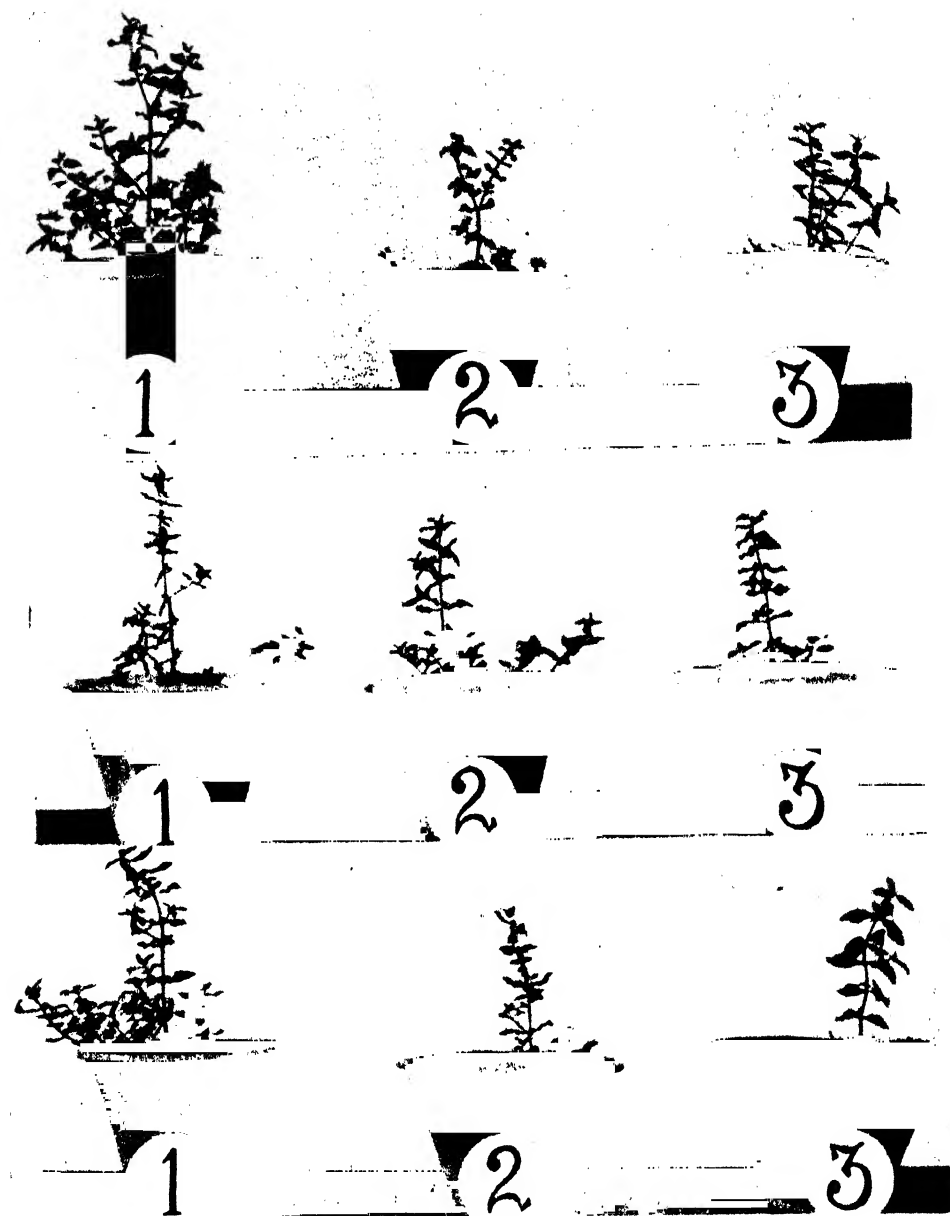


Fig. 1. Plants that are kept water-logged for a few minutes daily in the morning and moderately watered in the evening. $\times \frac{1}{2}$ of natural size.

Fig. 1.1—Plant growing in clayey soil.

Fig. 1.2—Plant growing in clayey loam.

Fig. 1.3—Plant growing in sandy loam.

Fig. 2. Plants that are moderately watered twice daily. $\times \frac{1}{2}$ of natural size.

Fig. 2.1—Plant growing in clayey soil.

Fig. 2.2—Plant growing in clayey loam.

Fig. 2.3—Plant growing in sandy loam.

Fig. 3. Plants that are moderately watered once daily in the morning. $\times \frac{1}{2}$ of natural size.

Fig. 3.1—Plant growing in clayey soil.

Fig. 3.2—Plant growing in clayey loam.

Fig. 3.3—Plant growing in sandy loam.



Plants grown in soils with different levels of Fig. 1, exchangeable calcium in the soil (1 of natural size). Figs. 1.1, 1.2, 1.3 and 1.4 show plants grown in soils with 12.0, 15.0, 24.0 and 36.0 me./l. of exchangeable calcium respectively.

The following conclusions are evident from this experiment. Firstly, *E. alba* prefers a clayey soil. The sandy loam is least suited for its growth as is evidenced by both the low seed output and the low fresh and dry weight of the shoots. Secondly, the best performance in respect of seed output in each set is obtained in the pot subjected to water-logging for a few minutes daily (Pl. X, Fig. 1). However, a clayey soil appears to be a more essential requirement for this species than the amount of water received as the plant growing in clayey soil moderately watered once a day gives a better performance than that of any of the other pots filled with clayey loam or sandy loam. However, the necessity of a minimum supply of moisture for the growth of the species is not to be underrated. A clayey soil with abundant moisture supply is the most favourable habitat for this species and in nature only under such conditions is the species to be found during the hot and dry months of summer.

Experiment No. 2 :—From the soil analysis data set in Table I, it is seen that the plant is found to have a wide range of tolerance for calcium in the soil, being able to thrive in calcareous as well as non-calcareous soils.

To assess how far calcium in the soil is responsible for the performance of the plant, culture experiments were undertaken. Four culture pots were filled with unmanured garden soil. Pot No. 1 was kept as an unmanured control and to the other pots lime was added. The soil analysed at the end of the culture experiment (after about $3\frac{1}{2}$ months) gave the following levels of exchangeable calcium in the soils of the culture pots:

Pot No. 1—Control with 12.0 m.e. % of exchangeable calcium			
Pot No. 2—Soil with 15.0
Pot No. 3— 24.2
Pot No. 4— 36.0

The detailed soil analysis data are given in Table 5.

TABLE V
Analysis of soils in culture pots

Soil analysis	Culture pots			
	1	2	3	4
pH	8.0	8.2	8.6	8.6
Carbonate content (%)	1.21	3.05	4.58	6.92
Exchangeable calcium (m.e. %)	12.0	15.0	24.2	36.0

Two plants were allowed to grow in each of the pots. The results obtained at the conclusion of the experiment are shown in Table VI (Pl. XI, Fig. 1).

It is seen that the plant thrives fairly well in all the culture pots. This confirms the field observations. However, the best performance is obtained in moderately calcareous soils. It is also seen that the value for root/shoot ratio decreases with increasing dosage of lime in the soil. In other words, root development with regards to shoot development is comparatively poorer in calcareous soils.

TABLE VI

Performance of E. alba in soils with different levels of calcium

Observations	Culture pots*			
	1	2	3	4
No. of heads	36	59	34	33
Av. No. of seeds per head	41	54	48	33
Seed output	1476	3186	1632	1089
Depth of root system (cm.)	30	29	39	55
Fresh wt. of shoot (g.)	10.370	16.510	10.020	9.020
Fresh wt. of root (g.)	2.412	3.120	1.520	1.205
Dry wt. of shoot (g.)	1.530	2.120	1.590	0.580
Dry wt. of root (g.)	0.240	0.300	0.210	0.200
Root/shoot ratio (fresh wt. basis)	0.233	0.189	0.152	0.134
.. .. (dry wt. basis)	0.157	0.142	0.132	0.127

* Pot contents :

Pot No. 1 - Control with 12.0 m.e. % of exchangeable calcium

Pot No. 2 - Soil with 15.0 m.e. % of exchangeable calcium

Pot No. 3 - Soil with 24.2 m.e. % of exchangeable calcium

Pot No. 4 - Soil with 36.0 m.e. % of exchangeable calcium

BIOTIC FACTORS

The plants are very susceptible to biotic influences. They are frequently met with in the grazing grounds of Varanasi. The growth is, however, stunted and prostrate. Fungal parasites are not reported on this species from India.*

PHENOLOGY

The plant is an annual and is found to thrive throughout the year flowering and fruiting freely. Flowering starts about two months after the seedlings appear and the plant completes its life in about 3 to 4 months. The incidence of the plant is maximum at the end of the rainy season and in winter. During the hot dry months of summer, the plants are restricted only to highly favourable habitats.

GERMINATION OF SEEDS

The seeds are found to have no dormancy period, but the germination percentage increases with storage as seen from Table VII. Seeds collected on the 1st September, 1957 were used in these experiments.

*This information was obtained from the Head of the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi.

TABLE VII

Germination of seeds of E. alba after dry storage for different periods

Date	No. of seeds germinated out of 50	Percentage germination
10-9-1957	2	4
2-11-1957	8	16
2-1-1958	31	62
2-7-1958	31	62

Salisbury (1929) has drawn attention to the importance of intermittent germination in the survival and establishment of seedlings in nature. In *E. alba*, the seeds which can germinate earlier will do so if the conditions are favourable and others later so that the chances of survival of seedlings are enhanced.

The germination behaviour of the seeds of *E. alba* was studied under different light conditions (Table VIII). The seeds were kept in (1) diffused day light and darkness at night, (2) continuous light of an electric bulb and (3) continuous darkness inside a chamber.

TABLE VIII

Germination of seeds of E. alba under different light conditions

Date	Number of seeds germinated out of 50		
	Diffused day light	Continuous light	Continuous darkness
3-1-59	0	15	0
4-1-59	0	1	0
5-1-59	0	3	4
6-1-59	3	4	1
7-1-59	1	1	1
8-1-59	7	0	0
9-1-59	1	1	0
10-1-59	1	1	0
11-1-59	1	0	0
12-1-59	1	0	0
13-1-59	2	0	1
14-1-59	2	1	0
15-1-59	2	1	0
16-1-59	3	0	0
18-1-59	0	12	0
20-1-59	0	1	0
22-1-59	1	0	0
23-1-59	1	0	0
24-1-59	1	0	0
25-1-59	1	0	0
26-1-59	1	0	0
27-1-59	2	0	0
Percentage Germination	62	82	14

From Table VIII it is seen that the maximum germination of 82 per cent is obtained in continuous light which may be partly due to the warm atmosphere around the electric bulb and also due to the direct effect of light itself. Very poor germination is obtained in the seeds that are kept in continuous darkness.

Fresh seeds collected on the 1st September, 1957 were buried under mud for four months and then put for germination in diffused day light. These seeds gave a germination of 72 per cent which is greater than that obtained from dry stored seeds.

REPRODUCTIVE CAPACITY

Salisbury (1942) defines reproductive capacity as the product of the average seed output and the fraction representing the percentage germination. It represents the intrinsic capacity of the species to reproduce. The reproductive capacity value for *E. alba* works out to 564. *E. alba* does not propagate itself by vegetative means though rooting at nodes is frequent, wherever they are in contact with the substratum.

EPIDERMAL STRUCTURE AND STOMATAL FREQUENCY OF LEAVES

The leaves have appressed hairs on both sides. The configuration of the epidermal cells of the lower and upper surfaces is different. The outline of cells of the upper surface is more or less straight in surface view, while those of the lower surface are wavy. Stomatal frequency and stomatal index values for this species in 'sun' and 'shade' leaves are set in Table IX.

TABLE IX

Stomatal frequency and stomatal index in 'sun' and 'shade' leaves of E. alba

		Upper epidermis		Lower epidermis		Stomatal index	
Sl. No.		Stomata per sq.mm.	Epidermal cells per sq.mm.	Stomata per sq.mm.	Epidermal cells per sq.mm.	Upper surface	Lower surface
Sun	I	255	686	373	824	24.7	31.2
	II	324	833	431	961	28.0	31.0
	III	275	706	402	912	28.0	30.6
Shade	I	196	608	225	411	24.4	35.4
	II	176	549	225	401	24.3	35.9
	III	196	618	225	401	24.1	35.9

It is seen that the number of stomata per unit area is more on the lower surface than on the upper surface. Moreover, the stomatal frequency is more in the open than in the shade.

OSMOTIC PRESSURE OF THE PLANT SAP

The osmotic pressure values for the plant sap of *E. alba* from different localities are shown in Table X. The osmotic pressure values were determined by cryoscopic method using a Beckman thermometer (Loomis and Shull, 1937).

TABLE X

Osmotic pressure of the plant sap of E. alba

Locality	Moisture content of substratum (%)	Osmotic pressure of plant sap (atmos.)
I	14.4	4.645
II	7.6	7.226
III	4.1	8.516

The values obtained for *E. alba*, as seen from Table X, are comparatively very low and are typical of a marshy plant. The osmotic pressure fluctuates according to the moisture content of the substratum.

DISPERSAL OF SEEDS

Ridley (1930) mentions of the following method of dispersal of seeds for this species. The surface of the achene is papillose and the fruit is sticky due to a mucilagenous exudation. Thus, the dispersal is possible by its adherence to the plumage and feet of birds. He attributes the appearance of the plant in Krakatau by this method. The human agency is also helping its dispersal either by adhesion to feet or clothes or to importation with other plants. He further mentions of seed drifts in water during floods though not quite satisfactorily as in the case of buoyant seeds.

SEEDLING MORPHOLOGY

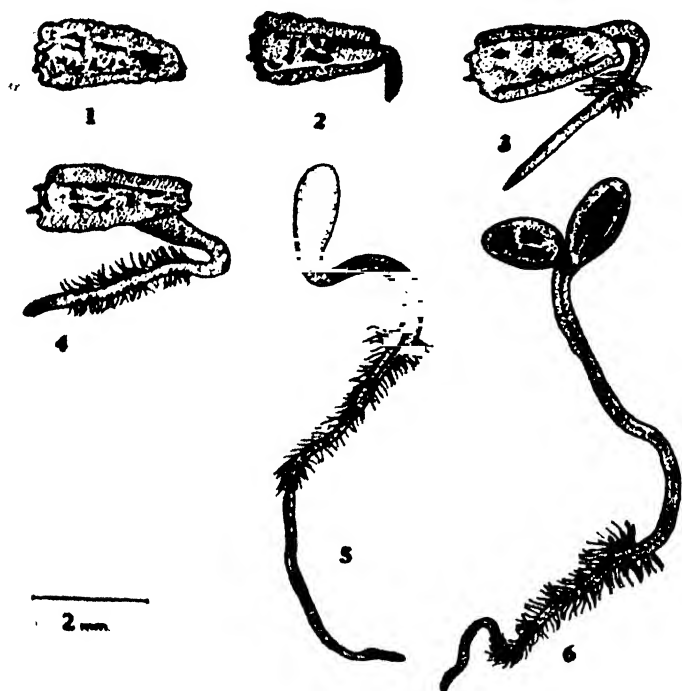
The germination of seeds in *E. alba* is epigeal. The radicle appears as a pro-truberance from the pointed end of the seed by a narrow split in the testa. When the radicle is a few mm. long, the hypocotyl elongates in the form of a bent. The two cotyledons are raised above the soil and in this process are withdrawn from the testa. The cotyledonary leaves expand and assume green colour (Text-fig. 1).

ECONOMIC IMPORTANCE

It is an old established Hindu medicine. Kirtikar *et al.* (1935) and Watt (1890) attribute the following medicinal properties for this species. The plant has a bitter sharp dry taste, good for the complexion of the hair, the eyes, the teeth; cures inflammation, eye diseases, 'kapha' and 'vata', bronchitis, asthma, leucoderma, anaemia, diseases of heart and the skin, itching, night blindness, syphilis, used to prevent abortion, miscarriage and uterine pains after delivery (Ayurveda).

It is used principally as a tonic and deobstruent in hepatic enlargements and in various chronic skin diseases. The fresh juice of the leaves rubbed on the shaven scalp promotes the growth of hair. The fresh plant is applied with sesamum oil in elephantiasis and the expressed juice in affections of the liver and dropsy. When used in large doses, it acts as an emetic. It relieves headache when applied with a little oil. The leaves are reputed to cure sores when applied to them.

In Indo-China, the plant is much used as a cure for asthma and bronchitis. The pounded leaves are prescribed in haemorrhage. In Ceylon, it is used to purify the blood.



TEXT-FIG. 1.

Stages in the germination of the seed.

Kirtikar *et al.* (1935) mentions about the occurrence of two varieties of the plant—the yellow flowered and the white flowered, the former variety have thicker leaves which are extensively used in catarrhal jaundice.

DISCUSSION

The ecological amplitude of a species is important in determining its presence in various habitats (Hanson, 1958). *Eclipta alba* Hassk. has a wide range of tolerance with regard to moisture level and calcium in the soil. In the case of *Euphorbia thymifolia* Linn. (in press), it has been found that the red form and the green form have different requirements of calcium in the soil, inasmuch as the former can tolerate a wide range of soil calcium whereas the latter can thrive only in calcium poor soils. In *E. alba* we find that though the plant can thrive in calcium poor soils, it has a poor seed output. In calcareous soils, on the other hand, fairly good performance is obtained in different levels of exchangeable calcium in the soil. However, the seed output value tends to be low in excessively calcareous soils. Steele (1955) has presented evidence to show that calcicoles are of at least two types, one being more exacting in its requirement than the other. Accordingly, *E. alba* can conveniently be grouped under the less exacting group of calcicoles. However, we find that a non-calcareous soil is not necessarily associated with an acidic reaction.

The necessity of soaking under mud, for germination, the seeds of *Mollugo hirta*, *Polygonum plebejum*, etc., has been shown by Mall (1954) to be an important factor in their occurrence as characteristic members of the drying pond bed flora. The seeds of *E. alba* are able to withstand waterlogging and in fact give a higher, percentage of germination after pre-treatment with mud.

The stomatal frequency of 'sun' leaves is found to be higher than that for the 'shade' leaves. Salisbury (1932) accounts for this as due to the drier environment obtainable in the open rather than to the direct effect of illumination.

ACKNOWLEDGEMENTS

The author is indebted to Prof. R. Misra, F.N.I., Head of the Department of Botany, Banaras Hindu University, for his valuable guidance, and encouragement during the course of this investigation.

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OSTEOLOGY OF *WALLAGO ATTU* BLOCH AND SCHNEIDER

PART I. OSTEOLOGY OF THE HEAD

by MRS. N. I. JOSEPH, *Department of Zoology, College for Women, Trivandrum*

(Communicated by E. S. Narayanan, F.N.I.)

(Received December 15, 1958; read March 6, 1960)

KUTCH MICROFAUNA—LOWER TERTIARY OSTRACODA

By B. S. TEWARI & K. K. TANDON, paper appears in

VOL. 26 (B), No. 4.

POSTSCRIPT—*Hermania* Puri, 1953 non Monterosato, 1844 is now included in the synonymy of *Hermanites* Puri, 1955 (Pokorny, Vladimir; 1958, *Grundzüge der zoologischen mikropaläontologie*, band II; p. 270). Consequently, the species *Harmia indica* and *Hermania purii*, n. spp. should now be referred to *Hermanites indicus* and *Hermanites purii* n. spp. respectively under the subfamily Trachyleberidinae Sylvester-Bradley.

Bhimachar (1933) and Gregory (1933) made a comparative study of the skull, the former author restricting his studies to certain Indian species. De Beer (1937) made a critical study of the homology of the bones of the skull and Eaton (1948) tried to correlate the form and function of the head in *Ictalurus*. Nawar (1954) gave a reasonably complete account of the osteology of *Clarias lazera*.

It is therefore clear that not much work has been done on the osteology of the head of Siluroidei. The complete osteology of only two species has been studied belonging to the families Amiuridae and Clariidae. As regards the family Siluridae to which *W. attu* belongs, very little has been done excepting a page description of the skull of *W. attu* by Bhimachar (1933) and a shorter account of

the osteology by Regan (1911). The complete osteology of the head of even a single species of this important family has not been worked out in spite of the many points of interest.

The author has therefore attempted to study in detail the osteology of the head of *W. attu*. The osteology of the remaining parts will be published as a series under the following heads :

Part II—Osteology of the vertebral column and associated ribs, weberian ossicles and median fins.

III—Osteology of the pectoral and pelvic girdles and paired fins.

IV—The lateral line ossicles.

MATERIAL AND METHODS

Over a hundred specimens of *W. attu* were examined ranging in length from 3 inches to over 3 feet. The collections were mostly made in person from the fishing centres of Changanacherry and Trivandrum. A few specimens over 2 feet were collected during the South-west monsoon flood from the Vembanad lake.

As a rule skeletons over 6 inches were prepared by the maceration process. Care was taken to locate the positions of the surface lying dermal bones and other bones which are loosely attached. They were subsequently replaced in situ on the dry skeleton by means of adhesive cement or by passing wires through the lateral line canal system wherever present.

A great advantage of this method of preparation was that the freshly prepared skeleton was still flexible and the degree of relative movement possessed by the different parts of the skeletal system could be studied.

The skeleton of specimens less than 6 inches was prepared by the alizarin technique and was used for the study of the position of surface lying dermal bones. Disarticulated skeletons were also studied.

OSTEOLOGY OF THE HEAD

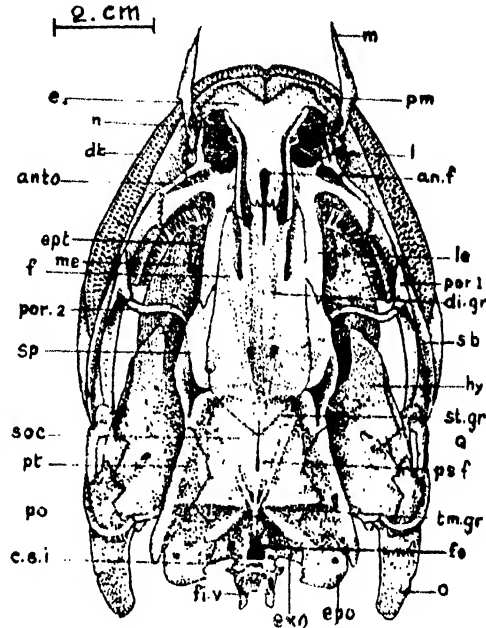
The head skeleton or the skull *W. attu* is a well ossified compact structure. The chondrocranium is ossified excepting small portions in the cranium. The main cartilaginous part is the posterior ethmoid region which remains unossified as the internasal septum. There are also cartilaginous surfaces on the lateral ethmoids* for the palatines. The preotics have an inner lining of cartilage and are separated from the exoccipitals by cartilage. Cartilage forms the floor and side walls of the foramen magnum and the epiotics have cartilage between the exoccipitals and pterotics. Most of the bones are well ossified, hard and are strongly connected with one another. The sutural connections formed by the interlocking of splint like processes are so intimate that they are in many cases invisible in the prepared skull. Many of the dorsal bones have shallow impressions on their dorsal surface.

Viewed from above, the skull is wedge shaped (T. Fig. I), with the point of the wedge directed anteriorly. The greatest width is in the auditory region and the length is about $1\frac{1}{2}$ times the maximum width. The side view (T. Fig. II) presents a triangular outline. The greatest height is in the region of the pre-opercular and this is about two times the length of the skull. The orbits are not well defined and the post-orbital process of the sphenotic is rudimentary. Posteriorly (T. Fig. III) there are seen the five processes characteristic of the teleostean skull—the two pterotic, the two epiotic and the supraoccipital spine.

* The terminology of bones included in this paper is according to De Beer (1937) unless otherwise stated.

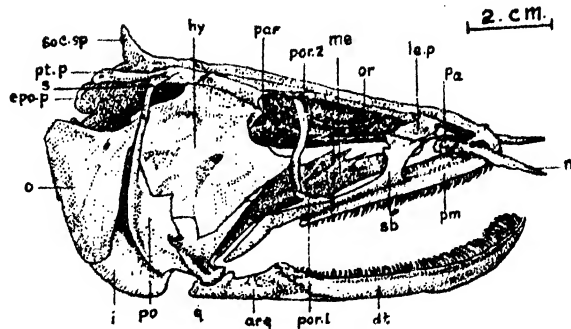
The skeleton of the head is composed of the following parts :

1. The neurocranium consisting of the cranium enclosing and protecting the brain and the sense capsules which protect the olfactory, optic and auditory organs.
2. The visceral arches and associated bones which form the jaws and the hyobranchial skeleton to support the gills.



TEXT-FIG. I.

Dorsal aspect of the skull (Hyoid cornua and Branchial arches removed)



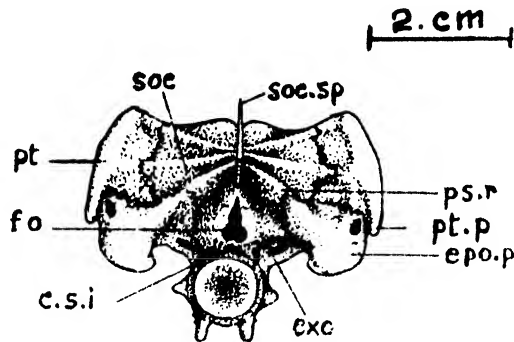
TEXT-FIG. II.

Lateral aspect of the skull (Hyoid cornua and Branchial arches removed)

1. THE NEUROCRANIUM

The neurocranium is platybasic (Kindred, 1919) as the cavum cranii extends widely up to the ethmoid region (T. Fig. IV). The mid-dorsal line of the cranium (T. Fig. IV) is not straight and the anterior 2/3 forms an angle of about 30° with the mid-ventral line. The mid-ventral line of the cranium (T. Fig. IV) is perfectly straight and the posterior region forms the deepest part.

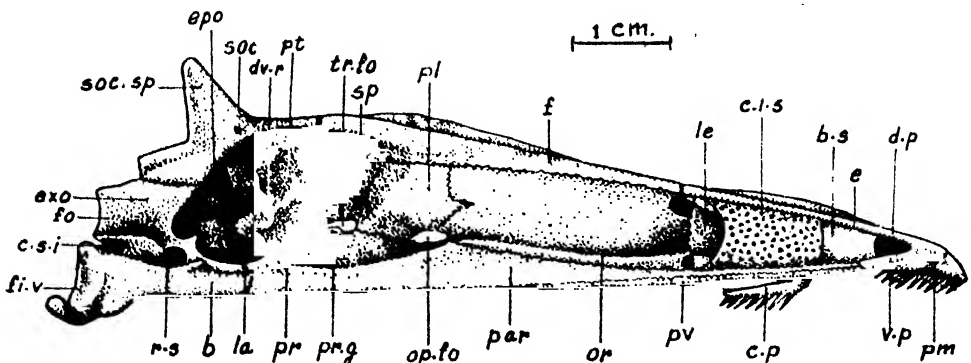
There are two narrow fontanelles in the roof of the cranium along the mid-dorsal line. The anterior fontanelle (T. Fig. I, an. f.) is between the ethmoid and the anterior region of the frontals. The posterior fontanelle (T. Fig. I, ps. f.) is between the posterior region of the frontals and the supra occipital.



TEXT-FIG. III.

Posterior aspect of the skull (Hyaloid cornua and Branchial arches removed)

The dorsal surface of the cranium is flat, sloping forwards and slightly backwards. The anterior end—the ethmoid region—is broadly obtuse. The width then sharply decreases in the preorbital region to provide space for the orbit and then again gradually increases beyond the orbit till the posterior extremity of the neurocranium. Hence the lateral edges beyond the orbits are slightly divergent.



TEXT-FIG. IV.

Median aspect of the bisected skull (Hyaloid cornua and Branchial arches removed)

From the midlateral region of each frontal a shallow groove arises—the dilator groove (T. Fig. I, di. gr.)—which runs outward towards the sphenotic and lateral ethmoid and terminates. There are two other grooves—the temporal groove

(T. Fig. I, tm. gr.) and the supratemporal groove (T. Fig. I, st. gr.) which are deeper. The more lateral of the two—the temporal groove—is the deeper and consists of three portions—an anterior portion in the anterolateral supraoccipital and anteromesial pterotic regions, a middle portion in the postero-lateral supraoccipital, postero-mesial pterotic and anterior epiotic, and a posterior portion in the posterior supraoccipital, anteromesial epiotic and anterodorsal exoccipital regions. The supratemporal groove is anterior and inner to the temporal groove. It is shallower and originates from the mesial posterior region of the frontal. Anteriorly from the sphenotic it slants diagonally inwards and proceeds to the base of the supraoccipital spine, by which it is separated from its fellow of the opposite side.

The midventral line of the cranium is keeled and in the posterior region, due to the depth of the keel, two large triangular lateral surfaces are formed, one on each side, for the attachment of muscles. There are two shallow wide fossae—one on each side in front of the auditory chamber bounded by the pleurosphenoids, sphenotics, and posterior regions of the frontals and orbitosphenoid.

The posterior surface of the neurocranium is only a gradual slope from the supraoccipital backwards. It forms an obtuse angle with the dorsal surface of the skull.

The neurocranium is divided into the following regions :

The ethmoidal region.

The orbito-temporal region.

The otic or auditory region.

The occipital region which articulates behind with the vertebral column.

The Ethmoidal Region

This is the anteriormost region of the neurocranium situated in front of and at a lower level than the frontals. It consists of those bones developed in relation to the snout and nostrils and comprises of the following: the ethmoid, the lateral ethmoids, the nasals, and the prevomer (Plate XII, Figs. 1-4).

The *ethmoid* is a large irregular median bone with anterior lateral processes—ethmoid cornua—(Plate XII, Fig. 1, e.c.). Posteriorly it is bifurcated into two horizontal processes (Plate XII, Fig. 1, h.p.) that go to meet the frontals and are tucked beneath them. These processes can be traced as faint flat ridges up to the anterior end of the bone. The body of the bone is hollow and posteriorly it is divided into two lateral chambers by a thin bony septum (T. Fig. IV, b.s.). These two chambers unite with one another anteriorly and together form the anterior portion of the ethmoidal cavity. Thus, this region of the ethmoid may be said to be split into a dorsal and a ventral plate (T. Fig. IV, d.p. and v.p.) which form an angle of about 30° with one another at the anterior end. The bone is broad anteriorly and the ethmoid cornua project laterally as stout horns, forming part of the anterior wall and floor of the nasal capsule (T. Fig. I, e.). The dorsal surface of the bone is smooth, ventrally there is a small backward projection for articulation with the parasphenoid. Both the dorsal and ventral articular surfaces are highly split up into splint-like processes for articulation with the adjoining bones.

The ethmoid articulates with the premaxillaries anteriorly, with the parasphenoid, and prevomer ventrally, with the frontals postero-dorsally and the nasals and lateral ethmoids laterally.

The *Lateral Ethmoids*. These are paired bones situated one on either side of the ethmoid. Each is a stout bone and has a central body from which arises a stout lateral process called the lateral ethmoid process (Plate XII, Fig. 2, le.p.) or the antorbital process. There is another less stout but longer process situated posterior to the former and called the postero-lateral ethmoid process (Plate XII,

Fig. 2, ps.l.p.). These two lateral processes of the lateral ethmoid make an angle of about 40° between them and form the anterior and mesial boundaries of the orbit. Postero-mesially between the ethmoid, frontals and lateral ethmoid, there is an unossified region for the passage of the ophthalmicus superficialis. In the centre of the dorsal surface of the body of the lateral ethmoid there is a foramen for the exit of the ophthalmicus profundus (Plate XII, Fig. 2, fo.o.p.) which enters the bone through another ventromesial foramen. At about the centre of the ventral surface of the body of the bone is a small foramen through which a vein draining the ventral surface of the snout enters the orbitonasal canal and leaves the same through another foramen situated just outer to the foramen for the entry of the ophthalmicus profundus. At the antorbital angle of the lateral ethmoid are two foramina, one above the other. The dorsal foramen is for the entry of the artery supplying the olfactory capsule and the ventral foramen for two of the branches of the ophthalmicus profundus. The main body of the bone is scooped out mesially to form an inner dorsal and ventral plate thus forming the roof, floor and outer wall of the passage for the olfactory nerve which enters the nasal capsule through the olfactory foramen. Anterior to the origin of the lateral ethmoid process, there is a conspicuous antero-dorsal concavity—the posterior wall and floor of the olfactory capsule (Plate XII, Fig. 2, o.c.). At the posterior extremity of the nasal capsule is a large foramen—the foramen orbito-nasale—which leads into the orbitonasal canal. This leads into a spacious chamber in the body of the bone and also serves as passage for a vein draining the anterior region of the snout, an artery supplying the nasal capsule and anterior region of the snout and also for two twigs of the ophthalmicus profundus. The lateral ethmoid process bears a small stout anterior process (Plate XII, Fig. 2, an.p.) near its tip for articulation with the antorbital and suborbital. Outer to the concavity forming the posterior region of the olfactory capsule is a partially ossified stout anterior articular surface for articulation with the lachrymal and palatine (Plate XII, Fig. 2, ar.l. and pa.).

Dorsally the lateral ethmoids articulate with the ethmoid. The nasals lie over them. Ventrally there is articulation with the prevomer and orbitosphenoid. Posteriorly there is a firm interdigitation with the frontals and sphenoids by means of the postero-lateral ethmoid processes. Laterally there is a double flexible union with the suborbitals and a loose articulation with the antorbitals by means of the lateral ethmoid processes. Anteriorly there is a loose articulation with the lachrymals and a ligamentous union with the palatines.

The two lateral ethmoids do not meet each other along the mid-dorsal line and the posterior roof of the ethmoid is incomplete. Thus an anterior fontanelle is formed, situated between the two posterior processes of the ethmoid and the frontals. This region forms the posterior portion of the ethmoidal cavity and there is a cartilaginous internasal septum (T. Fig. IV, c.i.s.) in continuation with the anterior bony septum which here divides the posterior ethmoidal cavity into right and left portions and forms the boundaries for the olfactory passages.

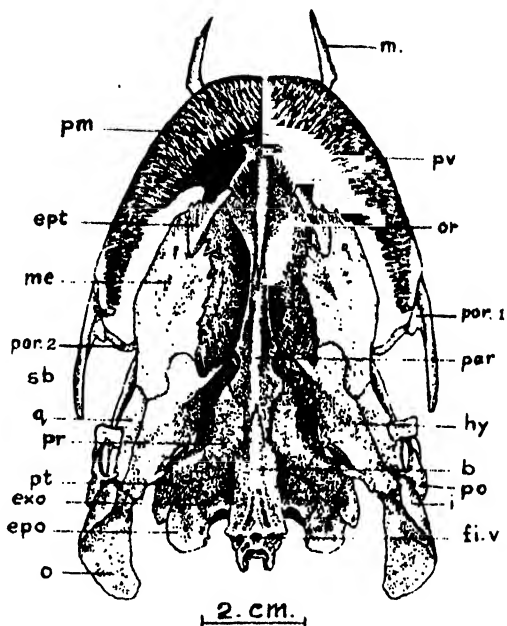
Both the ethmoid and lateral ethmoids are not traversed by any portion of the lateral line sensory canal.

The *Nasals*. These are two narrow long tubular bones lying one on either side of the ethmoid and over the lateral ethmoids, anterior to the frontals. They are not directly connected to the cranium but lie embedded in the connective tissue. The whole bone is traversed by the supraorbital portion of the sensory canal (Plate XII, Fig. 3 sor.c.). There is a small flat lateral process (Plate XII, Fig. 3, l.p.) near the anterior tip where the anterior end of the supraorbital canal bifurcates (Collinge, 1895).

The *prevomer* is a ventro-median "T" shaped bone situated below the ethmoid. At the junction of the head with the body of the bone, there are two elongated conical processes (Plate XII, Fig. 4 c.p.) directed backwards and outwards and set

with numerous minute conical teeth—the vomerine teeth—placed in shallow sockets. On the dorsal surface of the head of the bone there are two shallow lateral depressions one on each side. These form part of the floor of the nasal capsule. The body of the bone extends backwards below the orbito-sphenoid to meet the parasphenoid with which it is united by means of a prominent interdigitation. The middorsal surface of the prevomer forms part of the floor of the ethmoidal cavity.

The prevomer is united antero-dorsally with the ethmoid, antero-laterally with the lateral ethmoids and postero-dorsally with the parasphenoid (Plate XII, Fig. 4, ar. par. and T. Fig. V, pv.). Posteriorly the body of the prevomer lies in a groove below the parasphenoid.



TEXT-FIG. V.

Ventral aspect of the skull (Hyoid cornua and Branchial arches removed)

The anterior tip of the ectopterygoid is united by a strong ligament to a small vertically flat protuberance placed dorsal to the origin of the process carrying the vomerine teeth.

There is no rostral bone or cartilage. The space between the two anterior lateral horn like projections of the ethmoid is filled with connective tissue.

The Orbito-temporal Region

The orbito-temporal region is sub-divided into :

- a. The orbital region.
- b. The temporal region.

a. The Orbital Region

The orbital region consists of the bones that go to form the orbital ring. The orbits are large in size and lie in the anterior half of the neurocranium in the dorsolateral aspect and occupy about $\frac{1}{3}$ of its length. Since the neurocranium

is platybasic, the orbits are separated mesially by a narrow portion of the cranium. Each orbit is bounded anteriorly and dorsally by the lateral ethmoid, mesially by the prevomer, orbitosphenoid frontal and parasphenoid and postero-dorsally by the autosphenotic. Part of the ventrolateral and posterior boundaries are formed by the orbital bones, the lachrymals, the antorbitals, the suborbitals and the two post-orbitals (T. Fig. I, II and Plate XII, Figs. 5-9) formed in relation to the infraorbital branch of the sensory canal.

The lachrymals. Each is a small triangular bone lying in front of the antorbital and just over the anterior edge of the lateral ethmoid with which it is loosely articulated. The anterior angle of the bone is drawn out into a long spine like process (Plate XII, Fig. 5, sp.p.) which meets the ethmoid cornu thereby forming the outer lateral wall of the nasal capsule. Its posterior surface forms a small part of the anterior boundary of the orbit. The infraorbital branch of the sensory canal traverses the base of the bone emerging out at the inner posterior corner.

The antorbitals. These are small tubular bones found posterior to the lachrymals and anterior to the lateral ethmoid processes. They are united in front with the lachrymals (Plate XII, Fig. 6, ar.l.) and behind with the suborbitals and lateral ethmoids.

The suborbitals. These are long sturdy 'S' shaped bones found movably articulated with the lateral ethmoid process and lying posterior to the antorbital. They are about $\frac{1}{2}$ the length of the neurocranium and are traversed by the infraorbital branch of the sensory canal (Plate XII, Fig. 7 ior. c.) in its thicker anterior half. They are broader at the two ends and lie parallel to the long axis of the neurocranium extending from the lateral ethmoid process to the posteroventral angle of the neurocranium. The middle region lies firmly apposed to the posterior region of the premaxillaries. Each suborbital is articulated dorsally to the lateral ethmoid (Plate XII, Fig. 7, ar.le.) and to the antorbital and below to the premaxillary.

The first postorbitals. Each is a small flat roughly triangular bone found above the dorsal midregion of the suborbital to which it is loosely articulated by its base. It lies embedded in the connective tissue and is also loosely articulated to the second postorbital above. The infraorbital branch of the sensory canal is continued into it along its dorsal border from the second postorbital (Plate XII, Fig. 8, ior.e.).

The second postorbitals. These are slightly curved slender tubular bones situated between the sphenotics and the first postorbitals. They form the posterodorsal boundary of the orbit and is traversed by the sensory canal throughout their length. Each lies just beneath the surface of the skin and is loosely attached to the lateral edge of the sphenotic above and to the first postorbital below. The infraorbital branch of the sensory canal is continued into it from the sphenotic (Plate XII, Fig. 9, ior.e.).

b. The Temporal Region

The temporal region is subdivided into :

- b*₁. The frontal region.
- b*₂. The sphenoidal region.

*b*₁. The Frontal Region

The frontal region lies posterior to the ethmoid region between it and the sphenoidal region. It is formed by the frontals and the orbito-sphenoid (Plate XII, Figs. 10 and 11).

The *frontals* are a pair of flat thin bones found between the ethmoid in front and the supraoccipital behind. They form the largest part of the dorsal surface

of the neurocranium and occupy more than $1/3$ of its length. The dorsal surface is sculptured to form the supra-temporal and dilator grooves (Plate XII, Fig. 10, di. gr., st. gr.). From the outer lateral angle of each there arises a canal which passes diagonally inwards to the median posterior region at a point about $1/3$ the length of the bone from the posterior end. This is the supraorbital sensory canal (Plate XII, Fig. 10, sor.c.) which is continued into the nasal in front. At this point the sensory canal emerges from the bone and lies exposed in a groove giving rise to two branches at two different points which unite again before entering the sphenotic. Further behind, the sensory canal unites with its fellow of the opposite side and continues up to the posterior end of the frontals as a deep groove formed by the two frontals between them.

The two frontals do not unite along the anterior middorsal line for about more than $\frac{1}{2}$ their length, thus forming the posterior $2/3$ of the anterior fontanelle. In this region the frontals dip down vertically and mesially forming the side walls of the fontanelle. Posterior to this, the two frontals unite for a short space and then again diverge slightly forming the anterior half of the posterior fontanelle.

The frontals unite anteriomesially with the ethmoid, antero-laterally with the lateral ethmoids, and posterolaterally with the sphenotics. The posterior edge of the nasals lie over the anterior edge of the frontals outer to the ethmoidal suture. Posteriorly they unite with the supra-occipitals and posteroventrally with the pleurosphenoids. The frontal is a well ossified somewhat translucent bone.

The *orbitosphenoid* (Plate XII, Fig. 11). It lies below the frontals and forms the floor and side walls of the cranium in this region. The two lateral walls directed upwards do not unite with each other but unite with the downwardly directed mesial posterior portions of the frontals, thereby contributing to the side walls of the anterior fontanelle. The side walls are thinner, almost leaf like, when compared to the ventral portion of the bone. The ventral portion of the bone is broadened out and there is a groove on the ventral surface for receiving the elongated rod-like portion of the parasphenoid. The orbitosphenoid articulates anteriorly with the lateral ethmoids (Plate XII, Fig. 11, ar.le.) by means of two patches of interdigitation, dorsally with the frontals, ventrally with the parasphenoid and posteriorly with the pleuro-sphenoids (Plate XII, Fig. 11, ar. pl.).

b₂. The Sphenoidal Region

The sphenoidal region (T. Fig. I and IV) includes the parietals, pleuro-sphenoids and the parasphenoid. In *W. attu*, the parietals are absent (Goodrich, 1930). Their place in the formation of the roof of the skull seems to be taken up by the anterior region of the supraoccipital.

The *pleurosphenoids*. They are irregular curved bones situated between the sphenotics above the parasphenoid below and contribute to the formation of the posterior boundary of the optic and anterior boundary of the trigemino-facial foramen. Each forms the anterior lateral wall of the cranium in this region. The inner surface is concave and smooth while the outer surface is ridged forming the ridge found between the ventrolateral angle of the sphenotic and the optic foramen. The pleurosphenoids unite anteriorly with the frontals and orbitosphenoid, ventrally with the parasphenoid, ventrolaterally with the prootics and dorsolaterally with the sphenotics (Plate XII, Fig. 12, ar. f., ar. par., ar. pr., ar. sp., ar. or.).

The *parasphenoid* is a median, ventral, elongated, dagger shaped bone. The anterior elongated part of the parasphenoid which lies between the prevomer and orbitosphenoid reaches up to the ethmoid. Posterior to the orbitosphenoid the bone extends laterally upwards to form the characteristic wings of the teleostean parasphenoid.

The parasphenoid articulates anteriorly with the ventral plate of the ethmoid by means of splint like processes, dorsally with the orbitosphenoid and prootics, ventrally with the prevomer and posteriorly with the basioccipital (Plate XII, Fig. 13, ar.e., ar.or., ar.pr.; ar.b.). The posterior end of the prevomer overlaps the anterior end of the parasphenoid for about $\frac{1}{2}$ the length of the latter and there is an anteroventral recess on the parasphenoid for the reception of the body of the prevomer.

The *myodome*. Both the anterior and posterior myodomes are absent in *W. attu*. There is not even a myodomie space, (Bhimachar, 1933).

The *trigemino-facial chamber*. In *W. attu* the trigemino-facial chamber is not well developed. There is no well defined pars ganglionaris and at best it is represented only by a spacious depression or ledge on the antero-dorsal aspect of the prootic. The anterior and posterior openings are large and about equal to the length of the chamber or ledge in this case. They have almost coalesced and this makes the dorsal and lateral walls of the chamber separating it from the cranial cavity very negligible.

The trigemino-facial chamber may in this case be better called the trigemino-facial foramen (T. Fig. IV, tr. fo.) as it is only an elongated oval foramen with a broad concave ventral ledge which corresponds to the pars ganglionaris (T. Fig. IV and Plate XII, Fig. 14, pr. g.) in other forms.

The Otic or Auditory Region

The otic region (Plate XII, Figs. 14—18) is situated between the sphenoidal and occipital regions. It is formed of the following four chondral bones—the prootics, the pterotics, the sphenotics and the epiotics.

The *prootics*. These are large flat irregularly angular bones forming the anterolateral wall and the major part of the floor of the auditory capsule. The bone is concave and the concavity is divided, by a dorsoventral ridge (Plate XII, Fig. 14, dv.r.) which slopes backwards, into two portions. The anterior part forms the ventral ledge of the trigemino-facial foramen or the pars ganglionaris. The posterior part lodges the utriculus with the lapillus (Plate XII, Fig. 14, dep.la. and Fig. 18, la.) inside it, in a thin transparent shallow depression and also the horizontal semi-circular canal. The dorso-ventral ridge itself has in its dorso-posterolateral region a tubular canal for lodging the anterior semi-circular canal.

The prootic unites with its fellow of the opposite side along the median ventral line over the parasphenoid, thus forming the ventral floor of the cranium in that region. It unites anteroventrally with the parasphenoid, posteroventrally with the basioccipital, anteriorly with the pleurosphenoid, posteriorly with the exoccipital, anterodorsally with the sphenotic and posterodorsally with the pterotic.

The *sphenotic*. These are irregular bones situated dorso-laterally just anterior to the prootic and between the pleurosphenoid and the frontals. The sphenotics are the anteriormost of the auditory bones forming the lateral edge of the roof of the cranium in this region. Each has a thick irregular spongy body and a flat horizontal anterior process. The dorsal surface is flat and smooth in contrast to the ridged irregular ventral surface. The lateral line sensory canal splits up into the supraorbital and infraorbital branches (Plate XII, Fig. 15, sor.e., ior.e.) in the sphenotic and thus there is a three rayed sensory groove in the posterior dorsolateral surface of the bone inner to the sphenotic ridge (Plate XII, Fig. 15, sp.r.). The outer ventrolateral surface of the bone has a groove for articulation with the hyomandibular. The antero-lateral angle of the bone is produced into a concave facet for accommodation of the spine like articular process of the hyomandibular. The ventral surface

has a conical convexity in the inner posterolateral corner of the bone. This forms the portion of the cranium above the trigeminofacial foramen. The posterior tip of the bone is thick and spongy and bears a faint shallow depression which is the antero-lateral wall of the canal enclosing the anterior vertical canal.

On the anterior or orbital face of the body of the sphenotics there is a well defined concavity or pit towards the mesial region, inner to the articular facet for the hyomandibular. From the bottom of this concavity there is often a foramen (or sometimes a series of foramina) leading to a canal in the body of the bone. This canal is spacious and is directed posteriorly upwards and outwards opening behind by means of another posterior dorsolateral foramen placed dorsal to the groove for articulation with the hyomandibular. This is apparently the vestige of the spiracular canal.

The sphenotic articulates anteriorly with the lateral ethmoid, antero-mesially with the frontal, posteromesially with the supraoccipital, posteriorly with the pterotic, ventrally with the pleuro-sphenoid and prootic; ventrolaterally with the hyomandibular (Plate XII, Fig. 15, ar.le., ar.f., ar.soc., ar.pt.).

The *pteroics* are also irregular bones found in the posterior dorsolateral region of the neurocranium. The upper surface is smooth and shows shallow depressions corresponding to the anterior and median portions of the temporal groove. The lateral dorsal edge is formed into a ridge (Plate XII, Fig. 16, pt.r.) on a line with that of the sphenotic. This ridge corresponds to the bifid pterotic process found in other fishes, Dharmarajen (1936). The posterior end of the pterotic ridge is at a lower level than the preceding portion. The pterotics form the posterior lateral portion of the auditory capsule and are traversed by the horizontal semicircular canal which perforates internally the lateral wall of the bone. Posteriorly the bone is traversed by the inner and outer branches of the lateral line sensory canal which unites in the pterotic ridge at about the middle of its length. It is then continued forward into the sphenotic as a single canal giving off the preopercular mandibular branch.

The pterotics adjoin the sphenotics anteriorly, the epiotic and exoccipitals posteriorly (Plate XII, Fig. 16, ar.sp., ar.soc., ar.epo.).

The *epiotics*. These are roughly semicircular bones forming the posterior corner of the auditory capsule. From its posterior end there arises a flat downwardly directed epiotic process (Plate XII, Fig. 17, epo.p.) the base of which is traversed by the inner branch of the sensory canal before passing on to the pterotics. Anteromesially the bone shows a small recess for the posterior vertical semicircular canal.

The epiotic unites laterally with the pterotic, anteriorly with the supraoccipital and mesially with the exoccipital (Plate XII, Fig. 17, ar.pt., ar.exo.). A small articular facet is present at the junction of the epiotic with the posterior end of the pterotic ridge. This facet is for the movable articulation with the epiotic limb (superior limb) of the post temporal (described along with the pectoral girdle in Part III of this series.).

The Occipital Region

This is the hindmost region of the neurocranium connected in front with the otic and frontal regions and behind with the first vertebra. It forms the posterior roof, wall and floor of the cranium and is pierced by the foramen magnum at about the centre of its posterior surface. This region consists of the following bones: a dorsomedian supraccipital, a ventromedian basioccipital and lateral exoccipitals (Plate XII, Figs. 19-21).

The *supraoccipital*. This is the largest of all the occipital bones. It is flat, forming the roof of the cranium behind the frontals and bears a broad median

posterior vertical occipital process, the *supraoccipital spine* which is about $\frac{1}{2}$ the total length of the bone. The dorsal surface of the bone is smooth but grooved forming the posterior portions of the temporal and supratemporal grooves. The supraoccipital does not form the dorsal boundary of the foramen magnum as that region is cartilaginous due to insufficient ossification between it and the exoccipitals. There is a dorsomedian ridge and the anterior $\frac{1}{3}$ of the bone is cleft in the median line giving rise to the posterior portion of the posterior fontanella (Plate XII, Fig. 19, ps.f.). The bone also shows three pairs of lateral ridges which arise from the base of the supraoccipital spine. These are the anterior, lateral and posterior supraoccipital ridges (Plate XII, Fig. 19, an.r., l.r., ps.r.) of which the last is the most prominent. The anterior ridge proceeds forwards and outwards and meets the angular ridge over the posterior region of the frontal and sphenotic as in *Silundia*, Bhimachar (1933). The lateral ridge goes outwards towards the central basal portion of the pterotic ridge and the posterior ridge goes backwards and outwards to meet with a similar ridge on the epiotic. The posterior supraoccipital ridge is traversed by the posterior vertical semi-circular canal.

On the ventral surface of the bone in the centre, there are two pairs of foramina one behind the other. The anterior and posterior pairs are for the passage of the ramus lateralis accessorius and the auditory nerve respectively. On the dorsal surface, on either side of the median ridge at the anterior region of the base of the occipital crest, there is a pair of prominent foramina for the exit of the ramus lateralis accessorius (Plate XII, Fig. 19, fo.l.a.).

The supraoccipital articulates in front with the frontals, laterally with the sphenotics and pterotics, posteriorly with the epiotics and ventrally with the exoccipitals with which it is only apposed, cartilage still persisting between the two (Plate XII, Fig. 19, ar.f., ar.sp., ar.pt., ar.exo., ar.epo.).

The *exoccipitals* are situated laterally on either side of the foramen magnum and meet each other along the ventromedian line above the cavum sinus imparis thereby forming its roof and the ventral and lateral boundary of the foramen magnum. Each is highly irregular in shape and shows a posterior, anterior, mesial and ventral aspects. Mesially there are two smooth depressions one above the other separated by a ridge. The dorsal depression leads to the foramen magnum (Plate XII, Fig. 20, fo.). The ventral depression is the cavum sinus imparis (Plate XII, Fig. 20, c.s.i.) containing the sinus impar. It leads from a deep recess closed posteriorly—the recessus sacculus (Plate XII, Fig. 20, r.s.) which lodges the sacculle and lagena containing the sagitta and asteriscus respectively (Plate XII, Fig. 18, sa., as.). The median ridge forms the floor of the foramen magnum and the roof of the cavum sinus imparis.

Anteriorly there is a large anterolateral concavity which forms the posterior boundary of the otic capsule and contains the root of the vagus nerve. Viewed posteriorly there is a broad backwardly directed lateral ridge with a large foramen at its base. This is the foramen for the vagus nerve. Slightly dorsal to this is a small foramen for the exit of the branchial branch of the glossopharyngeal nerve. Anteroventrally towards the ventral median line is another small foramen for the exit of the palatine branch of the glossopharyngeal nerve.

The exoccipitals show clearly, posteriorly, the two lateral occipital condyles for articulation with the first vertebra which in this case is fused to the basioccipital.

The exoccipitals articulate anterodorsally with the supraoccipital, posterodorsally and posteroventrally with the epiotics, anteroventrally with the pterotics, ventrolaterally with the pterotics and ventromesially with the basioccipital (Plate XII, Fig. 20, ar.soc., ar.b., ar.epo.). Below the occipital condyle there is a very small ventrolaterally directed process which unites with a similar process—the lateral process—of the basioccipital to form an articular facet for the lower limb of the posttemporal.

The *basioccipital* is a midventral bone forming the posterior end of the neurocranium. It is situated beneath the exoccipitals and is broad and thick posteriorly and thin, pointed and splintered anteriorly. As mentioned before it is fused with the ventral half of the first vertebra and has at its posterior end two ventral posteriorly directed processes (Plate XII, Fig. 21, ps.p.) which fuse with similar processes of the first vertebra and form articulations with the second as well as the complex vertebra. Anterior to this on each side is a slight concavity bounded in front by a small stout lateral process. This is the articular facet for the lower limb of the posttemporal (Plate XII, Fig. 21, ar.pst.).

The anterior third of the bone is tucked between the parasphenoid and the prootics. Posterior to this the bone is thicker and on the dorsal surface there is a median ridge separating two smooth shallow lateral depressions. These are closed posteriorly and form the floor and mesial walls of the recessus sacculi. The median ridge is concave posteriorly forming a well marked groove which corresponds to the floor and side walls of the cavum sinus imparis (Plate XII, Fig. 21, fl. c.s.l.).

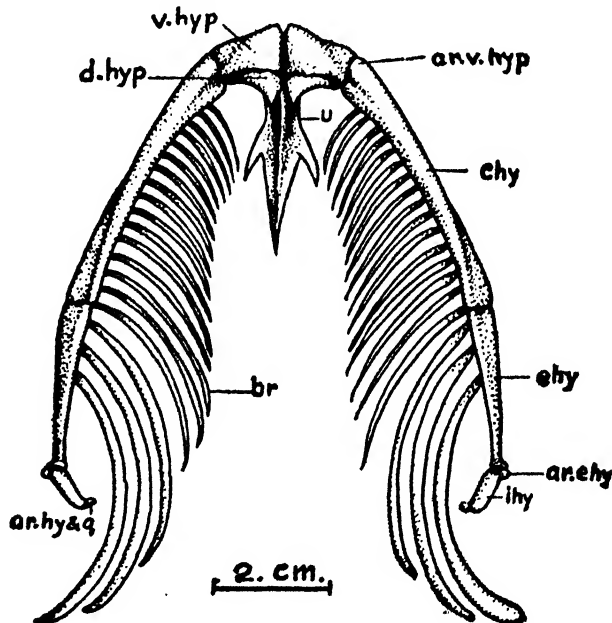
The basioccipital articulates anteriorly with the parasphenoid, antero-dorsally with the prootics, postero-dorsally with the exoccipitals and posteriorly with the posttemporals (Plate I, Fig. 21, ar. par., ar. pr., ar. exo., ar. pst.).

2. THE VISCERAL ARCHES

The visceral arches lie mainly in the pharyngeal wall internal to the coelom and they encircle the buccal and pharyngeal cavities. They are described as follows:

The mandibular arch or the first visceral arch. This forms the skeleton of the palate and gives rise to the secondary jaws or the jaws of the adult (Plate XII, Figs. 22-29).

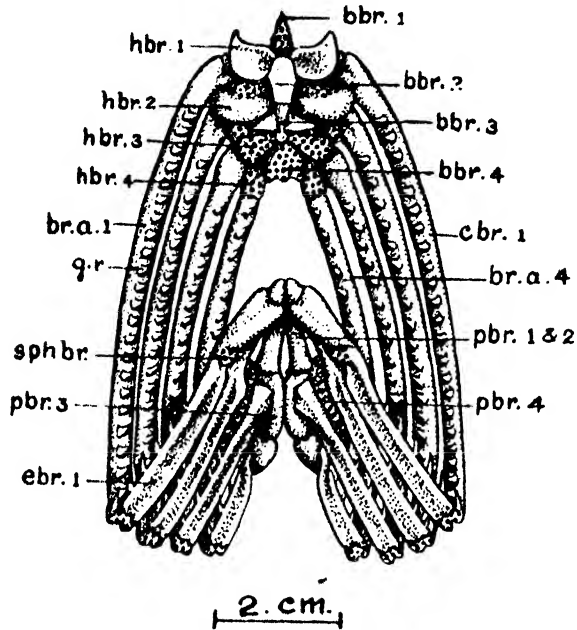
The hyoid arch or the second visceral arch. This forms the suspensorium and the hyobranchial skeleton (hyoid cornu) (Plate XII, Figs. 30-34 and T. Fig. VI).



TEXT-FIG. VI.

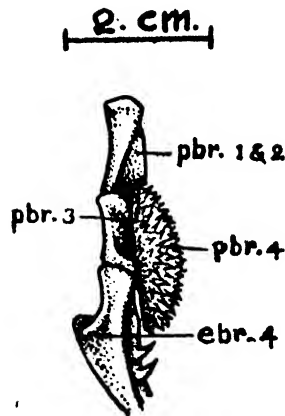
Hyoid cornua and associated bones

The branchial arches. The third to the sixth visceral arches from the four branchial arches which support the pharyngeal walls and the gills (T. Fig. VII and VIII).



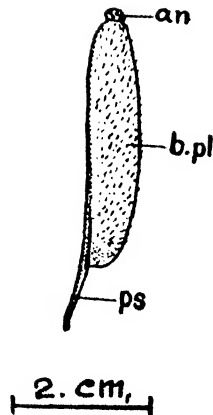
TEXT-FIG. VII.

Branchial arches and associated bones



TEXT-FIG. VIII.

Fourth Pharyngobranchial



TEXT-FIG. IX.

Infrapharyngeal bone

The infapharyngeal bones. The seventh visceral arch is incomplete and is represented on each side by a single bone, the infrapharyngeal bone, which bears teeth for pharyngeal mastication (T. Fig. IX).

The Mandibular Arch

The primary upper jaw is formed of three chondral bones on each side—the palatine, the metapterygoid and the quadrate. A paired dermal bone—the ectopterygoid—gets attached to this arch. In the adult all the above bones form only the skeleton of the palate and part of the suspensorium. There are in addition two more paired dermal bones—the premaxillary and the maxillary—of which the former alone bear teeth and take part in the formation of the adult upper jaw. The maxillary is highly modified for the support of the maxillary barbel.

The primary lower jaw formed of paired Meckel's cartilage persists as small rods of cartilage inside the adult or secondary lower jaw. Paired dermal bones, the angulars and the dentaries form the adult lower jaw.

The *premaxillaries*. They are thick curved bones which unite with each other anteriorly in the middle line. They form the anteriormost bone in the skull and are broad anteriorly but tapering posteriorly. Each is flattened dorsoventrally and the ventral surface is set with numerous irregular rows of sharp conical teeth in sockets. The teeth are directed backwards, the larger ones towards the inside. The premaxillaries alone form the gape of the mouth. The dorsal surface has the lateral margins slightly elevated. There are two slight irregular protruberances near the anterior end on either side of the shallow concave articular surface for the ethmoid (Plate XII, Fig. 22, ar.e.).

The premaxillaries are united anteromesially with the ethmoid, and posteriorly with the suborbitals for about half their length. They have a strong ligamentous connection with the maxillaries. The palatines are apposed to them on the dorsal surface behind the maxillaries. The anterior dorsal surface of the premaxillaries between the ethmoid and the maxillaries form the floor of the nasal capsule. The skull is akinetic since the upper jaw is immovably fixed to the braincase.

The *maxillaries* are small rod like bones placed parallel to the long axis of the skull over the dorsal surface of the premaxillaries external to the ethmoid. Each has a double groove along its length on the ventral aspect and the anterior end is pointed and dorsally forms a sheath for the cartilage of the maxillary barbel. The posterior end is flattened from side to side and expands into a double convexity which fits into shallow depressions found on the anterior surface of the palatine forming an incipient gliding joint that allows lateral movement.

The maxillaries are ligamentously united to the premaxillaries ventrally and also mesially to the protruberances outer to the ethmoid. Posterolaterally they are movably articulated to the palatines (Plate XII, Fig. 23, ar.pa.).

The *palatines*. These are very small irregular bones placed above the anterior dorsal surface of the premaxillaries between the maxillaries and the lateral ethmoids. Anteriorly they form a gliding movable articulation with the maxillaries (Plate XII, Fig. 24, ar.m.) and posteromesially they have a strong ligamentous attachment to the lateral ethmoids. The posterior surface is irregularly concave for the attachment of muscles. The anterior spinous angle of the lachrymals lies apposed to them dorsally.

The *ectopterygoids*. These are small flattened bones found anterior to the metapterygoids and placed obliquely. There is a narrow rod like anterior portion and a broad flat and slightly dorsally concave posterior portion. They form the anterior roof of the palate. The ectopterygoids are ligamentously attached anteriorly to the prevomers just dorsomesial to the process carrying the vomerine teeth (Plate XII, Fig. 25, ar.pv.). Posteriorly they are loosely united to the metapterygoids (Plate XII, Fig. 25, ar.me.). The endopterygoids are absent.

The *metapterygoids* are large irregular flat bones placed almost vertically and parallel to the long axis of the skull. Anteriorly the metapterygoids are loosely articulated to the ectopterygoids, posteriorly they are firmly united with the quadrates and the hyomandibulars (Plate XII, Fig. 26, ar. ept., ar. hy., ar. q.).

The *quadrates* are more or less irregularly triangular bones found anterior to the preoperculars and below the hyomandibulars. The lower angle is thick and has an articular facet which is concave in the transverse direction and convex in the longitudinal. This articular facet is lined with cartilage and gives articulation to a corresponding surface at the posterior dorsal surface of the articulars. From the posterior lateral margin of the articular facet, there is a small splintered posterior process (Plate XII, Fig. 27, ps.p. and ar. po.) for articulation with the preoperculars. Anteriorly, the bone is divided into an inner and an outer limb; the former articulates with the metapterygoid and the latter with the antero-ventral portion of the dorsally directed free limb of the hyomandibular (Plate XII, Fig. 27, ar. me. and ar. hy.).

The quadrates articulate ventrally with the angulars (Plate XII, Fig. 27, ar. ang.) posteriorly with the preoperculars, dorsally with the hyomandibulars and anteriorly with the metapterygoids. As the quadrates are immovably articulated to the neighbouring bones, the skull is monimostylic.

The *Angulars*. These are elongated bones, anteriorly thin, pointed splintered and hollowed out and this portion of each bone is overlapped by the dentary. Posteriorly, the bones are thick and solid. The remnant of the Meckel's cartilage is seen as a thin rod lying in the hollowed out portion (Plate XII, Fig. 28, mec.). It is attached to the solid posterior mesial portion of the angular and is continued forward into the tubular canal present in the dentaries.

The angulars articulate anteriorly with the dentaries, posterodorsally with the quadrates (Plate XII, Fig. 28, ar. dt. ar. q.). The posterior end of the angular is ligamentously attached to the posterior ventral inner region of the preoperculars. The posterior lower surface of the angulars is traversed by the mandibular branch of the lateral line sensory canal.

The *dentaries*. These are curved elongated bones of uniform width forming about $\frac{2}{3}$ the length of the lower jaw and carrying numerous sharp conical teeth in sockets. There is a posterior deep 'v' shaped indentation on the inner aspect of the bones for the reception of the angulars. The v-shaped indentation is continued forwards as a tubular canal (Plate XII, Fig. 29, c.mec.) in which extends the anterior end of the meckel's cartilages. The dentaries articulate with each other along the middle line, the articulation is lined with cartilage. Posteriorly, there is an articulation with the angulars (Plate XII, Fig. 29, ar. ang.).

On the outer dorsal edge, all along the length of each bone is a shallow groove in which runs the external branch of the mandibularis of the fifth cranial nerve. The anterior half of the mesial ventral edge shows anteriorly a longitudinal depression for the attachment of muscles. The mandibular branch of the sensory canal runs as a closed canal throughout the length of the bone on the inner ventral surface, outer to the above mentioned depression and there are nine sensory canal foramina distributed along its course.

The working of the adult jaws. The adult upper jaw is formed only by the premaxillaries and they are strongly united together at the anterior median line without the intervention of a rostral. This union between them is further strengthened by their firm attachment to the median ethmoid, which acts as an incipient copula. They are also indirectly attached to the ethmoidal region at two other places by means of the maxillaries and the palatines to the anterior region of the lateral ethmoids and by means of the suborbitals to the lateral ethmoid processes. Posteriorly, they have no connection either with the quadrate or with the lower jaw, since their posterior ends stop short a little anterior to the angle of the

mouth. However, the suborbitals to which the maxillaries are firmly attached extend beyond the angle of the mouth as far as the quadrates, thereby strengthening this region without minimising the elasticity of the mouth (T.Fig.II).

The adult lower jaw is articulated to the ventral angle of the quadrate, by a strong incipient ball and socket joint which allows up and down movement. The posterior end of the lower jaw is firmly attached by a strong tendon to the ventral end of the interhyal and the posterior lateral surface of the epihyal. The mandibular adductor muscles are inserted on the inner surface of the posterior region of the lower jaw, anterior to the quadrate articulation. The posterior ends of the premaxillaries are connected to the posterior region of the lower jaw by strong elastic dermal tissue, just anterior to the insertion of the above muscles.

Since the upper jaw is immovably fixed to the cranium, the mouth is opened or closed solely by the movement of the lower jaw. Due to the elasticity of the dermal connection between the upper and lower jaws at the angle of the mouth, the animal is enabled to open its mouth very wide in order to gulp in large pieces of food.

The Hyoid Arch

Each half of the hyoid arch consists of two segments : 1. The dorsal hyomandibula and 2. the ventral hyoid cornu. The hyomandibula consists of a single bone, the hyomandibular as the symplectic is absent. The hyoid cornu is composed of the following bones in a descending series : the interhyal, the epihyal, the ceratohyal and the two hypohyals. The basihyals are absent. Four dermal bones usually get associated with the hyomandibulars. These are the preoperculars, the operculars, the inter-opercular and the subtemporal. The suboperculars are absent (Bhimachar, 1933). Other dermal ossifications are the branchiostegals and the ventromedian urohyal which are attached to the hyoid cornu (Plate XII, Figs 30-34, and T. Fig. VI).

The hyomandibulars. These are large irregular thin flat bones situated vartically between the auditory capsule above and the quadrates below. Anteriorly each bone has two limbs, an inner mesial limb articulating with the metapterygoid and an outer dorsally directed free limb (Plate XII, Fig. 30, in.ar.me. and o.l.). Dorsally, there is a thickened flat articular surface for articulation with the sphenotic and the pterotic and anterodorsally there is a small thick spinous process—the anterior articular process (Plate XII, Fig. 30, an.ar.p.)—which fits into an articular facet formed by the anteroventrolateral edge of the sphenotic with the pleurosphenoid. At about the middle of the posterior edge, there is a third, thick ball like articular process—the posterior articular process (Plate XII, Fig. 30, pr.ar.p.)—for articulation with the opercular. Both the inner and outer surfaces of the bone are smooth. The outer posterodorsal surface bears two muscle impressions of the mandibular adductor muscles. Below this is a shallow groove for the passage of the hyoideus facialis nerve (Plate XII, Fig. 30, gr.hy.fa.). Anterodorsally there is another deeper groove (Plate XII, Fig. 30, gr.hyo.fa.) leading by means of an orifice into a small tubular canal in the dorsal region of the bone, the other opening of which is situated on the inner dorsal surface of the bone. This canal is for the passage of the main ramus hyomandibularis facialis nerve. On the anterior inner ventral surface of the body of the bone is a small tubular canal for the passage of a vein draining the lower jaw.

The hyomandibulars articulate anteriorly with the metapterygoids, dorsally with the sphenotics and pterotics, posteriorly with the operculars, preoperculars, and ventrally with the quadrates. The articulations with the auditory capsule and the operculars are movable and these together with certain parts of the articulations with the quadrates are lined with cartilage.

The preoperculars. These are thin crescent shaped bones placed posterior to the hyomandibulars and the quadrates. In each, the posterior edge is thick and

convex. The anterior edge is thin, irregularly dentated and slightly folded on itself ventrally to give accommodation to the posterior mandibular ligement. The fold is external and so there is a channel like groove on the inner ventral surface, the posterior edge of which gives origin to a portion of the above ligament. The dorsal and ventral ends are pointed. The dorsal end lies apposed over the posterodorsal edge of the hyomandibular, while the ventral end is firmly interdigitated with the posterior process of the quadrate leaving a slit-like opening for the passage of the ramus mandibularis facialis. The bone is traversed by the preopercular branch of the lateral line sensory canal, which is divided into two ventrally below the middle region. The outer surface of the bone is ridged along the course of the inner sensory canal and indicates the limit of the origin of the mandibular adductor muscles. At about the middle line of the inner anterior surface there is a small oblique ridge bounding a shallow groove on its anterior side. The interhyal lies in this groove.

Anteriorly, the preopercular unites firmly with the hyomandibular and the quadrate; posteriorly it is loosely apposed to the opercular and the interopercular. The interhyal lies in an oblique groove in the middle of its inner surface (Plate XII, Fig. 31, ar. s., ar. hy., ar. q.).

The *operculars* are irregularly triangular bones placed posterior to the preoperculars. The apex of each bone is placed anterodorsally and is thick with a somewhat deep socket for articulation with the ball like posterior articular process of the hyomandibular (Plate XII, Fig. 32, ar. hy.). This joint allows lateral movement for opening and closing the opercles. The muscle—dilator operculi—takes origin from all over the outer surface of the opercular, and the fibres lie in the laminae of the bone. The apex of the opercular above the articulatory concavity is produced into a thick, blunt, anteriorly directed process for the insertion of the muscle levator operculi. On the dorsal inner surface of the bone, there is a low ridge running a little below and parallel to the dorsal edge. The muscle adductor operculi is inserted all long the inner surface of this ridge.

The operculars are united anterodorsally to the hyomandibulars and ventrally to the interoperculars (Plate XII, Fig. 32, ar. i.). The preoperculars are loosely apposed to it for some distance along the anterior edge.

The *interoperculars*. These are thin flat bones with a convex ventral edge and a concave dorsal edge. They are placed below the operculars between it and the preoperculars, and thereby occupy in part the position of the subopercular (of other fishes) which is absent here.

The interopercular is united to the opercular above and the preopercular in front (Plate XII, Fig. 33, ar. o., ar. po.) the union with both these bones being not very firm.

The *subtemporals*, (Bhimachar, 1933). These are small elongated tubular bones developed in connection with the dorsal region of the preopercular mandibular lateral line canal. They are situated between the middle of the lateral edge of the pterotics and the dorsal edge of the preoperculars with both of which they are loosely united (Plate XII, Fig. 34, ar. i., ar. pt.).

The *interhyals* (T. Fig. VI, ihy.) are small rod like bones found between the hyomandibulars and the epihyals and posterior to the quadrates. They lie in shallow grooves formed by the inner surfaces of the preoperculars and the quadrates, ventral to the orifice for the exit of the hyoideus facialis nerve. They are firmly united dorsally to the hyomandibulars and anteriorly to the quadrates (T. Fig. VI, ar. hy; and ar. q.). Their ventral ends have a concave articular facet for articulation with the epihyals (T. Fig. VI, ar. ehy.). This articulation allows up and down movement to the epihyals. Posteriorly they unite with the preoperculars and the posterior mandibular ligaments are partially attached to the ventral ends of these bones.

The *epihyals* (T. Fig. VI, ehy.). These are elongated flattened triangular bones occurring between the ceratohyals and the interhyals. The posterior end of each is pointed and the outer dorsal edge gives attachment to a portion of the posterior mandibular ligament. Anteriorly each epihyal is broad and adjoins the ceratohyal. Between the two bones is a distinct layer of cartilage, the middle region of which is interrupted by a strong interdigitation of splints arising from both bones. The splints are present only on the outer surface of the bones and hence the cartilagenous connection is continuous on the inner surface. The mesial and outer surfaces of the bones are smooth and a low ridge on the ventral edge gives attachment to the last four branchiostegals of which the anteriormost is found at the junction between the epihyal and the ceratohyal.

The epihyals are movably articulated to the ventral end of the interhyals. Anteriorly they are firmly articulated to the ceratohyals.

The *ceratohyals* (T. Fig. VI, chy.). The ceratohyals are elongated bones situated between the epihyals and the ventral hypohyals (T. Fig. VI, ar.v.hyp.). Each is double the length of a epihyal and narrower in the anterior middle region than at the two ends. The posterior end is broad (as broad as the adjoining end of the epihyal) and flat, while the anterior end is thick and cylindrical with a spongy concave articulatory facet for the reception of the outer end of the ventral hypohyal. The inner ventral edge of the bone has a low ridge, in continuation with that on the epihyal for the attachment of the remaining anterior branchiostegals, excepting for a small region at the very anterior end.

The *hypohyals*. There are two on each side, a dorsal and a ventral (T. Fig. VI, d. hyp., v. hyp.), situated anterodorsal and anterior to the ceratohyals respectively. The ventral hypohyal is by far the larger and longer of the two.

Each *dorsal hypohyal* is a small, thin, irregularly triangular bone found between the first hypobranchial and the ventral hypohyal. The dorsal hypohyal is separated from its fellow by the cartilagenous first basibranchial and between it and the first hypobranchial is a distinct thin layer of cartilage. A cartilagenous layer is also present between the hypohyal and the ceratohyal as a thin lining on the articulatory surface, but the two are united by slight interdigitations on the outer dorsolateral surfaces. The dorsal hypohyals adjoin the first hypobranchials behind and the ventral hypohyals in front.

The *ventral hypohyals* are solid irregular bones with a smooth rounded anterior surface and a concave posterior surface. Mesially each is united firmly with its fellow of the opposite side, a thin lining of cartilage intervening between the two. The posterior end is thicker and dips down ventrally forming an elliptic convex articular surface for uniting with the ceratohyal. Each ventral hypohyal unites dorsally with the dorsal hypohyal, posteriorly with the ceratohyal and mesially with the median urohyal and with its fellow of the opposite side.

The *urohyal* (Harrington, 1955). The urohyal is a median sagittal membrane bone situated posterior to the hypohyals (T. Fig. VI, u.). It is irregular in shape, with a dorsoventrally flattened trident shaped ventral base having a laterally compressed longitudinal lamina of bone along the dorsal middle line. The teeth of the trident shaped base are directed posteriorly, the middle tooth being more than twice the length of the lateral teeth. The narrow anterior end is thick and is articulated with the two hypohyals and the cartilagenous first basibranchial. The vertical lamina of bone is broad (as broad as $\frac{1}{3}$ the total length of the bone) and extends from the anterior end longitudinally along the dorsal middle line, stopping short a little anterior to the posterior tip of the middle tooth. The muscles from the pectoral girdle are inserted on either side of this dorso-median lamina.

The *branchiostegals*. There are 18 to 21 branchiostegals, 19 being the most common, and they are attached by their bases to the ventral edge of the epihyals

and ceratohyals (T. Fig. VI, br.). Taking a case where there are 19 branchiostegals, the first 15 are usually attached to the ceratohyal, the sixteenth is attached to the junction of the ceratohyal, and the remaining three, seventeenth to nineteenth, are attached to the anterior half of the epihyal.

The size of the branchiostegals decreases from behind forwards and the first branchiostegal is only about $\frac{1}{3}$ the size of the last branchiostegal. The branchiostegals are free distally but are interconnected by skin and muscles—adductores branchiostegalium. Each branchiostegal is compressed laterally and consists of a dorso-ventrally enlarged slightly bifurcated base and a free curved pointed ray. The bases are attached to a low ridge along the inner ventral edge of the ceratohyals and the epihyals.

The *suspensorium* in *W. attu* is of the methyostylic type, (De Beer, 1937), where the primary upper jaw is suspended not by its own processes but by the hyomandibula articulated with the auditory region. Due to the highly modified nature of the skull, the maxillaries have lost their normal connection with the quadrates. Hence the adult upper jaw is attached to the neurocranium only in the ethmoid region. However, the quadrate still retains its articulation with the hyomandibular. The adult lower jaw is suspended only by the hyomandibular through the intervention of the quadrate, as the symplectic is absent.

The Branchial Arches

Each lateral half of a branchial arch is made up the following four bones from above downwards: the pharyngobranchial, the epibranchial, the ceratobranchial and the hypobranchial (T. Fig. VII). The two hypobranchials are united in the midventral line by a median basibranchial, which acts as a copula. The branchial arches carry gill rakers on the dorsolateral surfaces of the ceratobranchials and the ventral surfaces of the epibranchials (T. Fig. VII, g.r.). Gill filaments are present on the ventral surfaces of the ceratobranchials and the dorsal surfaces of the epibranchials.

The *pharyngobranchials* (T. Fig. VII, pbr. 1 and 2). The first and second pharyngobranchials are united to form a single elongated bone anterior to the third pharyngobranchial. It is dorsoventrally flattened, the anterior end is thick and narrow. It is placed parallel to the long axis of the skull and unites anteriorly with the united mesial end of the first and second epibranchials, a layer of cartilage separating the two. Posteriorly, it unites firmly with the third pharyngobranchial.

The third pharyngobranchials are thickened spongy bones nearly as broad as long and situated posterior to the fused first and second pharyngobranchials. On each side the third epibranchial is articulated on the anterior dorsolateral surface of the bone, while the fourth epibranchial is articulated to its posterior end. The ventrolateral surface is articulated to the fourth pharyngobranchial.

The fourth pharyngobranchials (T. Fig. VII and VIII, pbr. 4) have slipped down from their original position behind the third pharyngobranchials (to which they still retain a connection) and are placed ventrolateral to the latter bone. Each has developed as a flattened dorsoconcave, oval, toothed bony plate. The teeth are placed in sockets and are found all over the bone, excepting for a very small region mesially. The largest teeth are found posterolaterally and decrease in size anteromesially. The whole arrangement serves to work against the inferior pharyngeal bone in order to keep a firm grip on the prey while swallowing. There is an oblique anteromesial groove on the dorsal surface for firm articulation with the third pharyngobranchial. The anterior end of the fourth epibranchial is feebly articulated to it posteromesially on the dorsal surface.

The *epibranchials* (T. Fig. VII ebr.) are situated obliquely horizontally on either side of the pharynx between the pharyngobranchials and the ceratobranchials.

The posterior ends are directed outwards and backwards. They are elongated rod like bones deeply grooved dorsally for the posterior $2/3$ of their length as a passage for the branchial arteries, veins and nerves. At about $1/3$ the length on the anterior dorsal surface, just anterior to the grooved portion, each epibranchial has a flattened mesial process for insertion of the four muscles, levatores arcum branchialium. There is a gradual increase in the size of these processes caudad.

The first and second epibranchials are constricted and united for $2/3$ their length anteriorly. The third and fourth epibranchials are constricted in the same region for about the same distance, this constriction being more pronounced in the third epibranchial. The constricted portions do not carry gill rakers and gill filaments. The epibranchials are tipped with cartilage at both ends and are a bit more than $2/3$ the length of their corresponding ceratobranchials to which they are articulated posteriorly. The united end of the first and second epibranchials articulates to the anterior end of the fused first and second pharyngobranchials, the articulation being tipped with cartilage. The anterior ends of the third and fourth epibranchials are articulated to the anterior dorsolateral and posterodorsal surfaces of the third pharyngobranchial respectively. These articulations are tipped with cartilage.

The *ceratobranchials* (T. Fig. VII, cbr.) are elongated rod like bones situated between the epibranchials and the hypobranchials. They are the longest bones in the branchial arches and are all alike. They form the entire free ventral portion of the branchial arches. Each ceratobranchial is slightly curved upwards at both ends and at the same time slightly deflected outwards just anterior to the middle of the bone. It is deeply grooved along the ventral surface for the passage of the branchial blood vessels and nerves, the groove becoming shallower at the two ends. The ceratobranchials are also tipped with cartilage at both ends and articulate anteriorly and posteriorly with their own hypobranchials and epibranchials respectively.

The *hypobranchials*. All the four branchial arches possess hypobranchials (T. Fig. VII, hbr. 1-4), but the third and fourth are comparatively reduced and unossified. Each first hypobranchial is a flattened semicircular piece of bone, with a pointed anterior edge. It is almost as broad as long and about $1/9$ the length of its own ceratobranchial. It is articulated anteriorly to the dorsal hypohyal, mesially to the second basibranchial, posteriorly to the second hypobranchial and laterally to its own ceratobranchial. All the articulations are lined with cartilage.

The second hypobranchials are also of the same shape but the anterior edge is concave. They are about the same size and the anterior edge has a lining of cartilage. Each is about $1/7$ the length of its own ceratobranchial. It articulates anteriorly with the first hypobranchial, mesially with the second and the third basibranchial, posteriorly with the third hypobranchial and posterolaterally with its own ceratobranchial.

The third hypobranchials are cartilagenous excepting for a core of ossification, and their boundaries are not well defined. Each is a small roughly triangular piece of cartilage articulating by its base with the second hypobranchial and its own ceratobranchial. The narrow apex is directed mesially and gives articulation to the third and fourth basibranchial. Posteriorly it is articulated to the fourth hypobranchial.

The fourth hypobranchials are also cartilagenous and are somewhat cylindrical in shape. The boundaries are ill-defined. They are smaller and the apex is more acute and directed anteriorly. They give articulation anterolaterally to the third ceratobranchials, anteriorly to the third hypobranchials, posteriorly to the fourth basibranchial.

The *basibranchials* (T. Fig. VII, bbr. 1-4) are median bones placed along the ventral middle line between the hypobranchials and act as a copula for uniting the right and left half loops of the branchial arches. The first basibranchial is an ill-defined flat conical piece of cartilage found between the dorsal hypohyals. Posteriorly it gives articulation to the second basibranchial.

The second basibranchial is a well ossified dorsally flattened elongated semi-cylindrical piece of bone with the two ends slightly enlarged. The flat dorsal surface broadens out a little in the middle of the bone. It articulates anteriorly with the first basibranchial, anterolaterally with the first hypobranchial, posterolaterally with the second hypobranchial and posteriorly with the third basibranchial.

The third basibranchial is an elongated bone with the posterior half narrow and the anterior half gradually broadened out to more than double the width of the posterior region. Anteriorly it articulates with the second basibranchial, anterolaterally it is slightly apposed to the second hypobranchial, posteriorly it articulates with the fourth basibranchial and posterolaterally with the third hypobranchial.

The fourth basibranchial is a broad roughly shield shaped piece of cartilage placed anterior to the infrapharyngeal bones. It articulates anteriorly with the third basibranchial and the third hypobranchials, laterally with the fourth hypobranchials and posteriorly with the infrapharyngeal bones.

The *gill rakers*. In *W. attu*, a predacious form, feeding on live prey which it swallows, the gill rakers have become modified into sharp conical teeth like structures which thereby help to retain a strong grip on the prey and prevent its escape while being swallowed. The gill rakers are superficial structures and are not fused to the bones on which they occur. In the dried up skull, they can be easily removed and leave only a slight impression to denote their attachment. They are found here only on the epi and ceratobranchials in one or two rows, i.e. an outer and inner row. A table showing the number of gill rakers, their disposition on the bones and their variation is given below :

Names of bones	First branchial arch	Second branchial arch	Third branchial arch	Fourth branchial arch
Epibranchial	6-7 outer	7-8 outer	5-6 outer 4-6 inner	6 outer
Ceratobranchial	21-22 outer and 2 inner	20-22 outer and 7-9 inner	20 outer and 13-15 inner	22-25 outer and 2 inner

The *gill filaments* are slender laterally compressed pointed ray like structures. They are found in two dorsal rows on the epibranchials and two ventral rows on the ceratobranchials i.e. one row on either side of the groove containing the branchial blood vessels and nerves. They are cartilagenous and their bases are fused together and also with their fellows of the neighbouring row for about 1/3 their length, thus forming a continuous strong protective ventral sheath for the afore-mentioned blood vessels and nerves. Gill filaments are found on the epi-, cerato- and hypobranchials. They decrease in size gradually anteriorly on the epibranchials and hypobranchials to about a minimum of 1/3 that of the longest gill filament of the same branchial arch.

A table showing the number of gill filaments on each of the segments of the branchial arches and their variation is given below :

Names of branchial segments	First branchial arch	Second branchial arch	Third branchial arch	Fourth branchial arch
Epibranchial	75-80	78-80	76	68
Ceratobranchial	136-140	130	120	116-118
Hypobranchial	15	23-25	26-30	34-36

The *first suprapharyngobranchials*. These are small insignificant cartilagenous pieces one on each side, arising from the dorsal region of the first epibranchials, just anterior to the flattened mesial process (T. Fig. VII. sphbr.).

The Infrapharyngeal Bones

The infrapharyngeal bones (T. Fig. IX) represent the ceratobranchial elements of the fifth branchial arch. The two infrapharyngeal bones lie adjoining each other anteriorly on either side of the ventral longitudinal middle line, posterior to the fourth basibranchial. Each bone is elongated and curved diverging outwards from its fellow posteriorly. The anterior and posterior ends (T. Fig. IX, an., ps.) are sharply demarcated and the anterior end is short, cylindrical and partly cartilagenous. The posterior end is thin and pointed and well ossified. It is $\frac{1}{3}$ the width of the anterior end but about double its length. In between the two ends, there is a mesial broad curved elongated toothed bony plate (T. Fig. IX, b.pl.) meeting its fellow anteromesially but diverging posteriorly. It extends for about the anterior $\frac{5}{6}$ of the length of the bone and overlies the narrow anterior end. The teeth are conical and sharp and placed in sockets. They cover the entire surface of the bony plate and are directed postero-mesially. The infrapharyngeal bone articulates anteriorly with the fourth basibranchial and anterolaterally with the fourth hypobranchial. Its posterior end is free.

DISCUSSION

The osteology of the head of *W. attu* presents a number of interesting modifications which can be correlated to its habits. The bones of the head are all well ossified and the articulations strong, many showing splinters and interdigitations. Such an arrangement is necessary to withstand the shock caused when it swallows or snaps at large prey or large articles of food. The dorsal surface of the skull is almost smooth and the bones arranged in a compact manner. This together with the wedge-like shape of the head enables the fish to dart after its prey. Usually in teleost fishes the angle of the mouth is bounded by the upper and lower jaws, but here, as the maxillaries are used for the support of the maxillary barbels and the premaxillaries stop far short of the angle of the mouth, the latter is formed by an elastic dermal tissue, which gives an increase to the gape of the mouth. This aspect has not been reported so far. As an adaptation to its carnivorous habits, the animal is fully equipped with several rows of backwardly directed teeth on many of the bones in the mouth. Thus the premaxillaries are very well developed and well armed so also the prevomers, the infrapharyngeals and the fourth pharyngobranchials are toothed. The branchial arches also show this adaptation with the gill rakers being modified into strong sharp teeth in two rows.

The cephalic shield, so well developed in forms like *Clarias lazera* (Nawar, 1954) with the help of the supraorbitals, the dermosphenotic and the posttemporals, is not formed in *W. attu*. This is because it is active in habits and more of a necktonic form. Hence the observations of Gregory (1933) on the cephalic shield in Siluroids is substantiated by this negative proof.

Even though *W. attu* has a comparatively large head skeleton yet the bones are very light, hence one does not find any bracing of the junction of the skull with the backbone, as noticed in *Clarias lazera* (Nawar, 1954) or *Arius sona*, *Arius sagore* and *Osteogencosus militaris* (Bhimachar 1933).

Rudiments of the temporal fossa have been described in *Amiurus* (McMurrich, 1884) and in *Macrones* (Bridge and Haddon, 1893 and Bhimachar, 1933). In *W. attu*, there is no temporal fossa but the posttemporal arcade is represented by the posterior supraoccipital ridge which joins with a corresponding ridge on the epiotics.

The relationship of the ophthalmicus profundus to the lateral ethmoids is reported here for the first time. Eaton (1948) in *Ictalurus lacustris punctatus* describes a ligamentous connection between the lateral ethmoid and the third orbital bone. No such connection has been noted in the form under discussion. The lateral ethmoids in *W. attu* show a posterior firm interdigitation with the sphenotics by means of the posterolateral ethmoid process. This is apparently a primitive feature as it is seen in *Silundia gangetica* (Bhimachar, 1933) a primitive catfish and not seen in the more specialised forms.

A vestige of the orbitonasal canal seen in certain other teleosts, for example, in *Otolithus ruber* (Dharmarajen, 1936) has been found in this form and seems to be reported for the first time in the Siluroidei.

In *Amiurus catus*, Mc Murrich (1884) and Kindred (1919), there is no articulation between the prevomer and the lateral ethmoids. In the form studied, there is a firm articulation; this relationship is also seen in *Clarias lazera* (Nawar, 1954) and *Rita buchanani* (Bhimachar, 1933).

There has been some confusion with regard to the terminology of the orbital bones; Mc Murrich (1884) and Bhimachar (1933) have not clearly differentiated the orbital bones. A comparison with the orbital bones in other less specialised teleosts in relationship to the surrounding bones shows that in *W. attu*, there is only one antorbital, a single suborbital and two postorbitals on each side. The second postorbitals are identified by their relationship to the sphenotics (Dharmarajen, 1936).

The side walls of the orbitosphenoid are thick, laminated and poorly ossified in *Rita buchanani* (Bhimachar, 1933) and it is partially tubular due to the fusion of the sidewalls dorsally in *Silundia gangetica* (Bhimachar, 1933), *Amiurus catus* (Mc Murrich, 1884) and *Clarias lazera* (Nawar, 1954). In *W. attu*, the bone is not tubular and the side walls are thin but well ossified. Its floor is very thick and together with the prevomer and the parasphenoid forms a brace for the floor of the cranium (Gregory, 1933).

Generally in teleosts the basisphenoid is a small median Y-shaped bone placed above the parasphenoid. Its presence in the Ostariophysi is the subject of much controversy. Kindred (1919) and De Beer (1937) have reported its presence in *Amiurus*. According to Sagemehl (1884) and Berg (1940) it is lacking in Ostariophysi. Bhimachar (1933) has reported this bone in all the catfishes he studied, including *W. attu*, and as being fused on its ventral side with the parasphenoid. Its presence or absence can be established only by embryological studies which are not included in this paper. An examination of the adult skull of *W. attu* shows no clear indication of its presence or fusion to the parasphenoid.

In the otic region there is no myodome, nor even a myodomie space. Hence it is clear that *W. attu* belongs to the group of higher catfishes where the myodome has been obliterated by secondary simplification of the region. The myodome is present in very primitive catfishes like *Silundia gangetica* (Bhimachar, 1933). A rudiment of the myodome has been described in *Amiurus catus* (Mc Murrich, 1884). The modification and almost complete obliteration of the trigeminofacial chamber in Siluroidei is clearly seen in *W. attu*. This also seems to be a case of secondary simplification of the region, as the presence of a rudimentary pars ganglionaris indicates that the chamber was present in ancestral forms.

The vestige of the spiracular canal in the sphenotic described in *Amia*, *Allis*, 1903) and in *Otolithus*, (Dharmarajen, 1936) is often seen in *W. attu*. This is the first report of this vestige in the Siluroidei.

The supraoccipital does not contribute to the foramen magnum. This is similar to the condition seen in *Rita buchmanii* (Bhimachar, 1933). The posterior foramen on the ventral surface of the supraoccipital is the larger one and it gives passage for the auditory nerve. In *Amiurus* (McMurrich, 1884) this foramen is the smaller and is for the passage for the ascending branch of the first spinal nerve. The supraoccipital articulates only with the neural spine of the complex vertebra as the first and second vertebrae (the latter is free) have no neural arches.

In *W. attu* the basioccipital is completely fused with the first vertebra; the line of fusion is clearly visible on the dried up skull. It articulates posteriorly only with the posttemporals and is excluded from the formation of the foramen magnum by the exoccipitals. Nawar (1954) in *Clarias lazera* and Bhimachar (1933) in *Rita buchmanii* have described an articulation of the basioccipital with the complex centrum.

The reduction in the size and number of pterygoids appears to be a measure of evolution in the Siluroidei, as in *Silundia gangetica*, a primitive form (Bhimachar, 1933) all the three pterygoid bones are present. In *Amiurus* (Mc Murrich 1884) there is only the metapterygoid and a vestigial ectopterygoid which is a mere nodule of bone. In *Clarias lazera* (Nawar, 1954) and also in *Rita buchmanii*, *Plotosus canius*, *Pangasius buchmanii* and *Macrones aor* (Bhimachar, 1933) the ectopterygoids are reduced. But the degree of specialisation among these forms could be analysed by the relationship of the ectopterygoids with the prevomer and the quadrate. In the primitive form *Silundia gangetica*, it has a firm articulation with the prevomer and is toothed, the dentition being continuous with that on the prevomer. In *Rita buchmanii* it loses the dentition, but retains its articulation with the prevomer. In *W. attu* it is reduced and has only a ligamentous connection with the prevomer and in *Amiurus catus* (Mc Murrich, 1884) it is vestigial. Highly specialised forms like *Arius sona* and *Arius sagore* (Bhimachar, 1933) have developed secondary, firmer articulations with the lateral ethmoids. In *Clarias lazera* (Nawar, 1954) the ectopterygoids are well developed. They do not have any relationship with the prevomer but show a firm interdigitation with the quadrate. A comparison with the condition in other teleosts seems to indicate that the relationship of the ectopterygoids with the quadrate, and the anterior distal end being free is the least specialised or the most primitive condition. Their relationship with the lateral ethmoids is the most specialised condition and that with the prevomer is the intermediate stage. Therefore, it seems safe to assume that the nature of the articulation of the ectopterygoids could also be used as a measure of specialisation in the Siluroidei.

In the hyoid arch, the symplectic is absent. Nawar (1954) has also suggested the same condition in *Clarias lazera*. Mc Murrich (1884) in *Amiurus catus* and Bhimachar (1933) in *W. attu* and certain other forms discussed by him, have reported the presence of a small rectangular symplectic cartilage persisting between the hyomandibular, quadrate and the preopercular. It is suggested that this is not

a symplectic cartilage, as the same amount of cartilage of the same shape persists in the same position in teleosts where there is a well developed symplectic, for example, in *Otolithus* (Dharmarajen, 1936). In the skull of certain fishes there is persisting cartilage between several bones and it therefore seems reasonable to assume that the small amount of cartilage persisting between the hyomandibular, quadrate and preopercular is homologous to the cartilagenous interspace in *Otolithus* (Dharmarajen, 1936). The position of the symplectic is completely filled up by the interhyal.

In the hyoid arch there is no basihyal (Nawar, 1954) but the urohyal acts as an effective copula in this region. The branchial arches show slight fusion between parts, and some segments are cartilagenous. But all the elements are represented. This is in contrast to a form like *Clarias lazera* (Nawar, 1954) where there is reduction of all segments except the ceratobranchials.

The homology of the bone carrying the dorsal patches of pharyngeal teeth has not been clarified so far. Mc Murrich (1884) named it as the epipharyngeals in *Amiurus*, but did not give the homology. Nawar (1954) called it the dorsal pharyngeal patches of teeth and stated that the bases of the teeth fuse to give rise to a concave bone. In *W. attu*, the dorsal patches of pharyngeal teeth are placed in sockets and are in no way different from those on the premaxillaries, prevomer, or infrapharyngeals and the bones carrying these pharyngeal teeth have definite articulations both with the third pharyngobranchials and the fourth epibranchial. A comparison with the condition in a less specialised teleost like *Otolithus* (Dharmarajen, 1936) shows that in the latter, the third, fourth and to a very small extent the second pharyngobranchials carry the dorsal patches of pharyngeal teeth, and also that there is a tendency for the posterior pharyngobranchials to monopolise this function. Hence in *W. attu* as the first to third pharyngobranchials are present, and as the bones carrying the dorsal patches of pharyngeal teeth have articulations with the third pharyngobranchials and the fourth epibranchials, it appears safe to conclude that they are the fourth pharyngobranchials which have slipped down to a more ventral position in order to ensure a better grip against the infrapharyngeals.

In conclusion the osteology of *W. attu* seems to indicate that it belongs to the group of higher catfishes due to the absence of the myodome and the nature of the ectopterygoids. But the absence of the cephalic shield, the presence of a deeply situated head skeleton, the presence of all elements in the branchial arches and the lack of secondary articulations between the skull and the vertebral column, indicate that it is not a very highly specialised form. The lightness, compact arrangement of bones, and almost smooth dorsal surface, the wedge like shape of the skull together with the elasticity of the gape of the mouth and the full complement of teeth make it well suited to its active, predaceous, necktonic life.

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LIST OF ABBREVIATIONS

<i>an</i>	Anterior end
<i>ang</i>	Angular
<i>an.ar.p</i>	Anterior articular process
<i>an.f.</i>	Anterior fontanelle
<i>an.p.</i>	Anterior process
<i>an.r.</i>	Anterior ridge
<i>ant.o</i>	Antorbital
<i>ar.ang</i>	Articular surface for the angular
<i>ar.b</i>	Articular surface for the basioccipital
<i>ar.dt</i>	Articular surface for the dentary
<i>ar.e</i>	Articular surface for the ethmoid
<i>ar.ehy</i>	Articular surface for the epihyal
<i>ar.epo</i>	Articular surface for the epiotic
<i>ar.ept</i>	Articular surface for the ectopterygoid
<i>ar.exo</i>	Articular surface for the exoccipital
<i>ar.f.</i>	Articular surface for the frontal
<i>ar.hly</i>	Articular surface for the hyomandibular
<i>ar.i</i>	Articular surface for the interopercular
<i>ar.l</i>	Articular surface for the lachrymal
<i>ar.l and pa</i>	Articular surface for the lachrymal and palatine
<i>ar.le</i>	Articular surface for the lateral ethmoid
<i>ar.m</i>	Articular surface for the maxillary
<i>ar.me</i>	Articular surface for the metapterygoid
<i>ar.o</i>	Articular surface for the opercular
<i>ar.or</i>	Articular surface for the orbitosphenoid
<i>ar.pa</i>	Articular surface for the palatine
<i>ar.par</i>	Articular surface for the parasphenoid
<i>ar.pl</i>	Articular surface for the pleurosphenoid
<i>ar.po</i>	Articular surface for the preopercular
<i>ar.pr</i>	Articular surface for the prootic
<i>ar.pst</i>	Articular surface for the posttemporal
<i>ar.pt</i>	Articular surface for the pterotic
<i>ar.pv</i>	Articular surface for the ligamentous attachment to the prevomer
<i>ar.q</i>	Articular surface for the quadrate
<i>ar.s</i>	Articular surface for the subtemporal
<i>ar.soc</i>	Articular surface for the supraoccipital
<i>ar.sp</i>	Articular surface for the sphenotic
<i>ar.v.hyp</i>	Articular surface for the ventral hypohyal
<i>as</i>	Asteriscus
<i>b</i>	Basioccipital
<i>bbr</i>	Basibranchial
<i>b.pl</i>	Bony plate
<i>br</i>	Branchiostegal
<i>b.s</i>	Bonyseptum
<i>br.a</i>	Branchial arch
<i>cbr</i>	Ceratobranchial
<i>chy</i>	Ceratohyal
<i>c.i.s</i>	Cartilagenous internasal septum
<i>c.mec</i>	Canal for remnant of meckel's cartilage
<i>c.p</i>	Conical process
<i>c.s.i</i>	Cavum sinus imparis
<i>dep.la</i>	Depression lodging the lapillus
<i>d.hyp</i>	Dorsal hypohyal
<i>di.gr</i>	Dialator groove
<i>d.p</i>	Dorsal plate
<i>dt</i>	Dentary
<i>dv.r</i>	Dorso ventral ridge
<i>e</i>	Ethmoid
<i>e.c</i>	Ethmoid cornu
<i>ebr</i>	Epibranchial
<i>ehy</i>	Epihyal
<i>epo</i>	Epiotic
<i>epo.p</i>	Epiotic process
<i>ept</i>	Ectopterygoid
<i>exo</i>	Exoccipital
<i>f</i>	Frontal
<i>fi.v</i>	First vertebra

<i>f.c.s.i</i>	Floor of the cavum sinus imparis
<i>fo</i>	Foramen magnum
<i>fo.l.a</i>	Foramen for the exit of the lateralis accessorius
<i>fo.o.p</i>	Foramen for the exit of the ophthalmicus profundus
<i>g.r</i>	Gill raker
<i>gr.hy.fu</i>	Groove for the passage of the hyomandibularis facialis
<i>gr.hyo.fu</i>	Groove for the passage of the hyodius facialis
<i>hbr</i>	Hypobranchial
<i>h.p</i>	Horizontal process
<i>hy</i>	Hyomandibular
<i>i</i>	Interopercular
<i>ihy</i>	Interhyal
<i>in.ar.mc</i>	Inner mesial limb articulating with the metapterygoid
<i>ior.c</i>	Infraorbital sensory canal
<i>l</i>	Lachrymal
<i>lu</i>	Lapillus
<i>le</i>	Lateral ethmoid
<i>le.p</i>	Lateral ethmoid process
<i>l.p</i>	Lateral process
<i>l.r</i>	Lateral ridge
<i>m</i>	Maxillary
<i>mec</i>	Meckel's cartilage
<i>me</i>	Metapterygoid
<i>n</i>	Nasal
<i>o</i>	Opercular
<i>o.e</i>	Olfactory capsule
<i>o.l</i>	Outer free limb
<i>op.fo</i>	Optic foramen
<i>or</i>	Orbitosphenoid
<i>pe</i>	Palatine
<i>par</i>	Parasphenoid
<i>pbr</i>	Pharyngobranchial
<i>pl</i>	Pleurosfenoid
<i>pm</i>	Premaxillary
<i>po</i>	Preopercular
<i>por</i>	Postorbital
<i>pr.</i>	Preotic
<i>pr.g</i>	Pars ganglionaria
<i>ps</i>	Posterior end
<i>ps.ar.p</i>	Posterior articular process
<i>ps.f.</i>	Posterior fontanelle
<i>ps.l.p</i>	Posterior lateral process
<i>ps.p</i>	Posterior process
<i>ps.r</i>	Posterior ridge
<i>pt</i>	Pterotic
<i>pt.p</i>	Pterotic process
<i>pt.r</i>	Pterotic ridge
<i>pv</i>	Prevomere
<i>q</i>	Quadrato
<i>r.s</i>	Recessus sacculus
<i>s</i>	Subtemporal
<i>sa</i>	Sagitta
<i>sb</i>	Suborbital
<i>soc</i>	Supraoccipital
<i>soc.sp</i>	Supraoccipital spine
<i>sor.c</i>	Supraorbital canal
<i>sp.</i>	Sphenotic
<i>sphbr</i>	Suprapharyngobranchial
<i>sp.p</i>	Spine like process
<i>st.gr</i>	Supratemporal groove
<i>sp.r</i>	Sphenotic ridge
<i>tm.gr</i>	Temporal groove
<i>tr.fo</i>	Trigeminal foramen
<i>u</i>	Urohyal
<i>v.hyp</i>	Ventral hypohyal
<i>v.p</i>	Ventral plate.

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THE REPTILIAN HEART

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ABSTRACT

Hearts of twenty nine species of reptiles are studied here with a view to understanding the relation between the incomplete inter-ventricular septum of the non-crocodilian reptiles and the complete inter-ventricular septum of the crocodiles. The pars muscularis of the crocodilian inter-ventricular septum has been shown to correspond to the incomplete inter-ventricular septum of the lower reptiles. The pars endocardialis has no counterpart within the ventricle of the lower reptiles. It is a new formation and has probably developed as a ventricular continuation of the left endocardial ridge of the embryonic bulbus. The 'secondary or vertical' septum of some authors is merely the most medial and prominent of the many vertical ridges present in the wall of the ventricle. It is unlikely that it plays any role in the completion of the inter-ventricular septum. It has been shown, on circumstantial grounds, that the incomplete inter-ventricular septum lies between the pulmonary arch and the systemic arches. The present study records the salient features of the morphology of the reptilian heart also.

INTRODUCTION

Reptiles are the first truly terrestrial vertebrates in so far as their respiration and reproduction are concerned, but in other respects they are still in the process of evolution and hence are imperfect. This is particularly so in regard to the circulatory organs. The heart of the reptile is, in most cases, imperfectly four-chambered. There are two auricles separated from one another by a complete interauricular septum while the single ventricle is divided imperfectly into two parts by means of a muscular partition which is still incomplete. It is only in the crocodiles that the heart becomes completely four-chambered. How this change from a three-chambered heart of the lower reptiles to a four-chambered one of the crocodile is brought about is a question that has been troubling the minds of the anatomists for a long time.

A scrutiny of the literature on the reptilian heart reveals that there has been some misunderstanding regarding the nature of the ventricular cavity and the septa therein.

Goodrich (1916) declared that "in the reptilia the interventricular septum tends to divide the chamber into a left cavity leading to the base of the right systemic arch and a right cavity leading to the base not only of the pulmonary but also of the left systemic arch". He also believed that the separation of the left and right cavities may have been brought about not by mere fusion of the incomplete muscular septum with the opposite wall, but by the growth from behind forwards of a new muscular septum differentiated from the muscular strands which unite the base of the old septum with the dorsal wall of the ventricle.

O'Donoghue (1918) opined that regarding Ophidia and Lacertilia the above conclusion needed modification and said that in these reptiles the ventricle is "partially divided into a right and left chamber but the two systemic arches come off from the right side and the pulmonary arch comes off from the left". With regard to the flow of blood within the heart he has caused some confusion by saying that "whereas in the Crocodilia and Chelonia as in birds and mammals the aerated

blood is poured into the left side of the ventricle, in *Ophidia* and *Lacertilia* the reverse is the case and the aerated blood passes into the right ventricular chamber".

Rau (1924) described, in addition to the incomplete interventricular septum, a median vertical muscular ridge dividing the caudal portion of the large dorsal cavity into right and left halves. This vertical ridge is also incomplete anteriorly.

Leene and Vorstman (1930) called this vertical muscular ridge as the "vertical septum" and attributed to it great phylogenetic significance. They believed that it helped in the formation of the complete interventricular septum of the crocodilian heart.

Mathur (1944) mentioned incomplete dorsal and ventral "septoid processes" occurring as the anterior remnants of the median vertical ridge. He, however, named the interventricular septum "muscular ridge" and homologised it with the median vertical muscular ridge described by Rau (1924).

Foxon (1955) emphasising the role of the median vertical muscular ridge said that "it is this secondary septum which in crocodiles and birds becomes the definitive interventricular septum". He has also stated that in mammals the interventricular septum is derived from the primary septum.

Regarding the relationship between the openings of the aortic trunks and the incomplete interventricular septum of the lower reptiles there is a tendency to separate *Crocodylia* and *Chelonina* from *Ophidia* and *Lacertilia*. Thus O'Donoghue (1918) states that the interventricular septum lies between the pulmonary and left systemic trunks in *Ophidia* and *Lacertilia* while it lies between the pulmonary and the right systemico-carotid trunks in *Crocodylia* and *Chelonina*. Goodrich (1919) describes the opening of the right systemic trunk as being dorsal to the interventricular septum and that of the pulmonary trunk ventral to it, the opening of the left systemic trunk being situated almost opposite to the free edge of the interventricular septum, nearer to the opening of the right systemic trunk in *Ophidia* and of the pulmonary trunk in *Chelonina*. von Hofsten (1941) also considers that the position of the left systemic trunk in relation to the ventricular septum cannot be ignored and observes that in *Lepidosauria* it is dorsal to the septum while in *Chelonina* and *Crocodylia* it is ventral to it.

Thus a review of the literature on the reptilian heart will bring to light the fact that our understanding of its internal structure, particularly of the ventricle, is far from being conclusive. It also shows that there is still no correct evaluation of the role that the incomplete interventricular septum and other structures within the ventricle play in the formation of the complete interventricular septum of the crocodilian heart.

It is this fact that induced the present writer to study this problem afresh from a comparative point of view and find out the true nature of the ventricular cavity and of the interventricular septum. For this study, hearts of twenty-nine species of reptiles were used. Most of these species belong to *Ophidia* and *Lacertilia*, those of *Chelonina* and *Crocodylia* being relatively few. A list of the names of reptiles studied is given at the end. Most of the specimens were collected at Dharwar and neighbouring areas in North Karnatak. A few specimens were procured from Bangalore, Annamalainagar, Waltair and Visnagar through the kind co-operation of friends to whom the author is greatly indebted.

The hearts were dissected under a stereoscopic binocular dissection microscope. Hearts for serial sectioning were fixed in Bouin's fluid and the sections were stained with Ehrlich's or Delafield's Haematoxylin. In some cases sections were counter-stained with eosin or Congo-red. Wax model reconstructions of some hearts were also made. Hearts of *Geomyda trijuga*, *Calotes versicolor* and *Naja naja* have been treated as representing *Chelonina*, *Lacertilia* and *Ophidia* for purposes of illustration. All the figures have been drawn by the author.

Shape and size of the heart

A detailed account of the external morphology of the heart of reptiles has been sent to press. Only pertinent features are herein recorded.

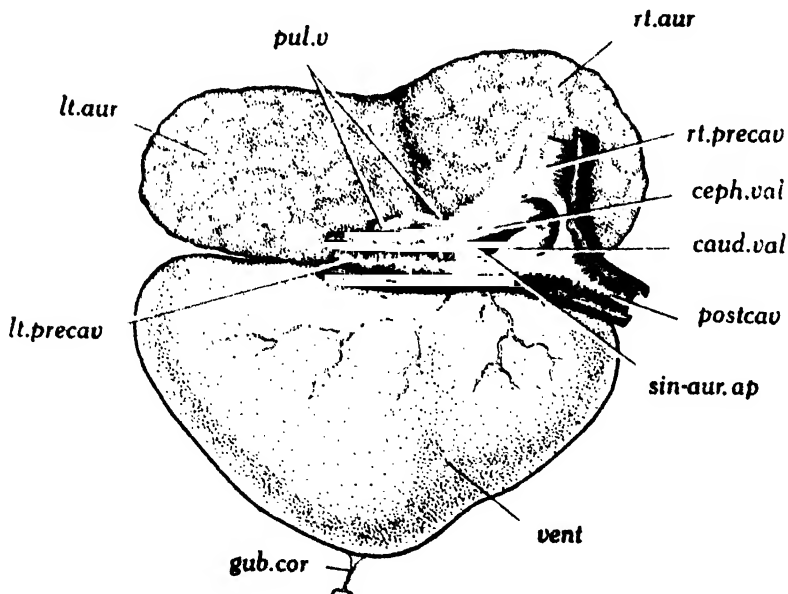
The shape of the heart seems to depend upon the external form of the animal to which it belongs. Thus in *Chelonia* the heart is generally broader than long, in conformity with the characteristic flat and laterally expanded body.

In *Rhynchocephalia* and *Lacertilia*, the heart is slightly broader than long and bears some resemblance to the chelonian heart in typically lizard-like forms such as *Sphenodon punctatus* (O'Donoghue, 1920), *Hemidactylus leschenaulti*, H. *flaviviridis* (Mahendra, 1942), *Calotes versicolor*, *Teratolepis fasciata*, *Ophisops beddomi*, *Mabuya carinata* and *Varanus monitor* (Mathur, 1944). Unlike the chelonian heart the apex of the lacertilian heart is nearly always pointed. In lizards in which the shape of the body is altered as in *Chamaeleon Zeylanicus*, *Riopa guentheri* (Kashyap, 1951) and *Barkudia insularis* the heart tends to become elongated. In *Barkudia insularis* in which the body is elongated and devoid of limbs, the heart is extremely modified and shows highly unequal auricles, a deep interauricular fissure, oblique coronary sulcus and even a left anterior shoulder-like extension of the base of the ventricle as in the heart of snakes.

In *Ophidia* the heart is highly elongated and exhibits all the characters referred to in connection with the heart of *Barkudia insularis*, but in a more marked and typical manner. The heart of *Acrochordus granulatus*, the marine Colubrid snake is, however, unique in being highly truncated in appearance and in being situated almost in the middle of the body as in no other snake so far studied.

The heart of crocodiles, like those of lizards and *Rhynchocephalia*, is only slightly elongated.

Regarding the size of the heart relative to the size of the body it is found that the chelonian and lacertilian hearts are generally bigger than those of other reptiles. Among the snakes, which generally show a small heart relative to the size of the body, *Eryx johni* seems to be an exception in possessing a comparatively large heart.



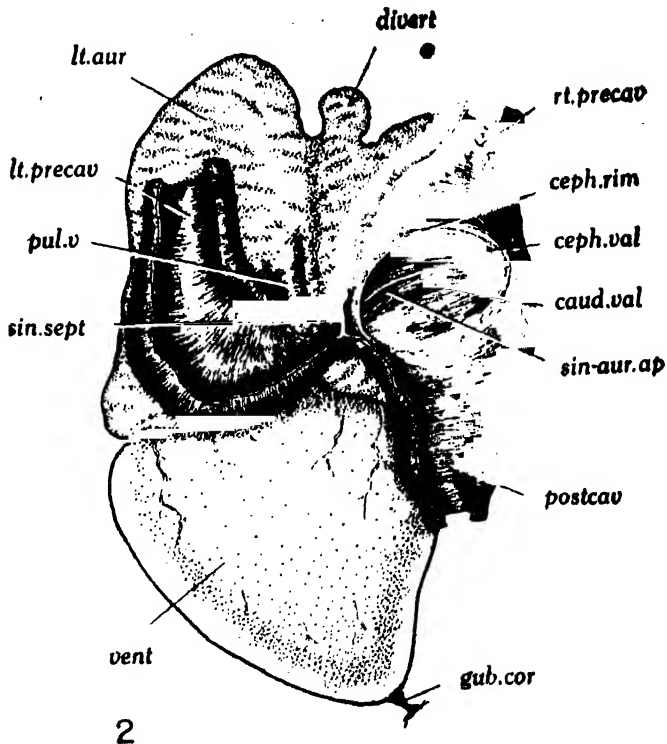
TEXT-FIG. 1.

Dissection of the snius venosus of *Geomyda trijuga* showing the oblique sinu-auricular aperture with the cephalic and caudal valves.

A gubernaculum cordis is generally present in the hearts of Chelonia, Lacertilia and Crocodilia.

Venous trunks and Sinus Venosus

The base of the venous trunks and the sinus venosus are usually of large size in Chelonia and Lacertilia. The internal structure of the sinus venosus is at its simplest in the chelonian heart, there being no sinus septum (Text-fig. 1). Among the lizards, there are some forms whose sinus venosus does not possess a well developed sinus septum. Such forms are *Hemidactylus flaviviridis* (Mahendra, 1942), *H. leschenaulti*, *Teratolepis fasciata*, *Riopa guentheri* (Kashyap, 1951), *Varanus monitor* (Mathur, 1944) and *Tiliqua scincoides* (Rau, 1924). Generally, however, the sinus septum is well developed in the lacertilian heart (Text-fig. 2). In those lizards in which it is absent, the junction between the left precaval vein and the sinus venosus does not show any constriction. In Ophidia, the structures inside the sinus venosus have undergone some reduction in conformity with the reduction in the size of the venous trunks and the elongation of the sinus venosus (Text-fig. 3). In the crocodiles, the sinus venosus is very much reduced, suggesting its ultimate disappearance in the hearts of birds and mammals.



TEXT-FIG. 2.

Dissection of the sinus venosus of *Calotes versicolor* showing a well developed cephalic rim at the junction of the sinus venosus with the dorsal wall of the right auricle. Note the large size of the venous trunks and the well developed sinus septum.

Auricles

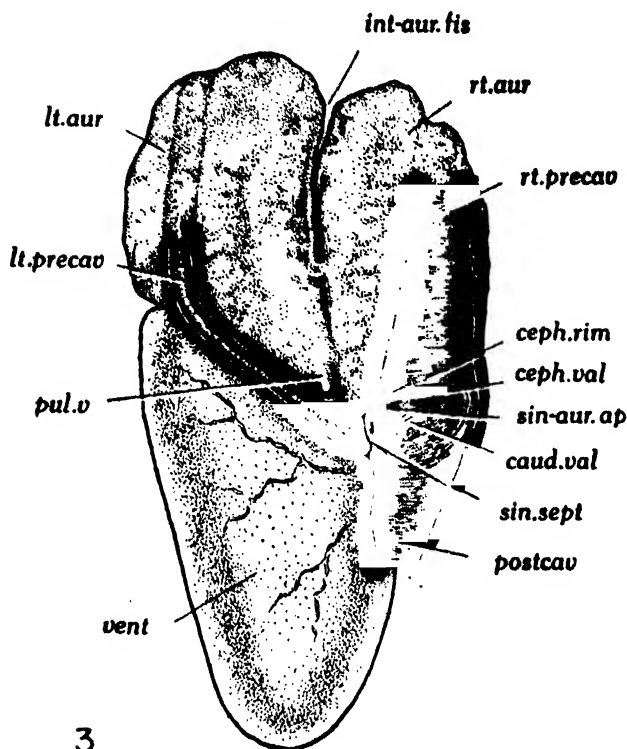
The auricles are thin walled and highly distensible. Hence when gorged with blood they appear darker and larger than the ventricle. The difference in size between the two auricles is not so emphasised in the chelonian heart. The difference is moderate in lacertilian, rhynchocephalian and crocodilian hearts while it is extreme in the ophidian hearts. Along with the increase in disparity in the size of the auricles and lengthening of the heart as a whole, the interauricular fissure is also seen to become deep.

Diverticulum

The presence of a diverticulum on the auricle does not connote any special significance, as its formation is a specific peculiarity brought about by the disposition of the aortic trunks or their immediate branches and the pressure they exert on the auricles. Generally, it is the right auricle that bears the diverticulum and the right pulmonary artery is often responsible for its formation. An exception to this is the heart of *Ptyas mucosus* (Ray, 1934) in which the diverticulum is borne on the left auricle and is caused by the left systemic arch.

Valves

Regarding the disposition of the sinu-auricular valves, there is a remarkable uniformity in the hearts of all lower reptiles. The sinu-auricular aperture and the sinu-auricular valves that form its anterior and posterior margins are both obliquely transverse (Text-figs. 1-3). The ends of the valves towards the left side extend into the base of the left precaval vein and slightly overlap each other, the cephalic valve being ventral to the caudal. Towards the right side, the two valves run vertical and parallel to one another and gradually fuse to form a ridge called the suspensory ligament. The auriculo-ventricular apertures are, each, guarded by a single large mesial valve and in some hearts by an additional small lateral valve (Text-figs. 4-6). The mesial valve is attached to the posterior end of the interauricular septum. Its dorsal and ventral margins are attached to the corresponding portions of the ventricular wall. The lateral margin alone is free, thin, notched and inflexed. It is this portion of the valve that plays an important part in preventing the regurgitation of blood during the ventricular systole. The mesial valve is bowl shaped, with the hump of the bowl towards the auriculo-ventricular opening and the cavity towards the ventricle. When the right valve is pushed towards the auriculo-ventricular aperture during the ventricular systole it is seen to fit snugly into a corresponding hollowness in the base of the ventricle, outside the rim of the auriculo-ventricular aperture. In such a position, the inflexed lateral margin of the valve opens out and lies just against the opening of the right systemic trunk. This fact is of considerable significance in directing the two streams of blood into the systemic arches, the mixed blood finding its way into the left systemic arch whose opening lies just beyond the limits of the lateral margin of the valve when it has opened out and the arterial blood finding its way into the right systemic arch, which is the only channel left open for the blood during the last phase of the ventricular systole. The right mesial valve is usually larger and stronger than the left. The mesial valves show a gradual increase in size and efficiency from *Chelonia* through *Lacertilia*, *Ophidia* and *Crocodylia*. In the last group, the openings of the aortic trunks are actually situated within the mesial valves, antero-mesially. Hence the valves act like funnels directing the blood into the aortic trunks, during the ventricular systole. The opening of the right systemic trunk is situated within the left mesial valve while those of the left systemic and pulmonary trunks are within the right mesial valve.



TEXT-FIG. 3.

Dissection of the sinus venosus of *Naja naja* showing the cephalic rim, sinu-auricular aperture with valves and the sinus septum. Note the oblique disposition of the left preceval and the elongation of the sinus venosus.

Cartilaginous support

A cartilaginous support is frequently present in the hearts of Chelonia, Lacertilia and Crocodilia. It is unifocal in the first two groups and multifocal in the last one. Ophidian hearts are usually devoid of a cartilaginous support. However, a small cartilaginous rod has been found in the heart of *Typhlops braminus* (Kashyap, 1950). The usual location of the cartilage is near the anterior end of the interventricular septum, in the vicinity of the pocket valves of the aortic trunks, sometimes extending slightly beyond the limits of the ventricle. The cartilage is generally of the hyaline type.

Ventricle

The ventricle is the most important part of the cardiac anatomy from the point of view of the shift from a three-chambered condition to a four-chambered one. In the ventricle of all lower reptiles there are two large cavities, a cavum dorsale situated antero-dorsally towards the left side and a cavum pulmonale situated ventrally towards the right. These two cavities are completely separated from one another in the caudal portion by the interventricular septum, while anteriorly, where the interventricular septum becomes incomplete, they communicate with each other freely. The present study has shown that the hearts of Chelonia, Lacertilia and Ophidia form a progressive series showing a gradual

increase in the thickness of the wall of the ventricle, particularly towards the left side (Text-figs 4-6). This brings about a corresponding reduction in the extent of the cavum dorsale. The thickened ventricular wall, towards the left side, is broken up into numerous vertical ridges which lodge spacious crevices among them which will trap the arterial blood as it rushes into the ventricle and hold it till the venous and mixed blood reach the pulmonary and left systemic trunks.

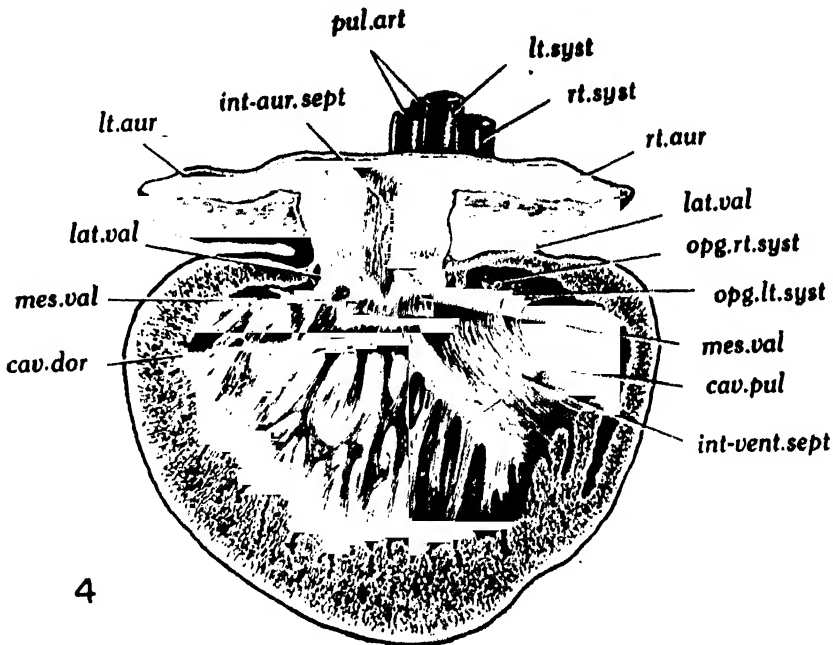
It should be noted that the classical view regarding the selective distribution of blood to the three aortic trunks in Amphibia and Reptilia has been questioned by some authors recently. Thus Vandervael (1933) and Foxon (1947, 1951 and 1955) have brought out experimental evidence to prove that mixing of blood takes within the amphibian ventricle. A similar view has been expressed by Prakash (1952) concerning the lizard, *Uromastix hardwickii*. However, Foxon and his associates (1956), using similar radiographical methods on another lizard, *Lacerta viridis*, have supported the selective distribution hypothesis. Simons and Michaelis (1953) using fluorescent dyes, have shown that in the heart of the frog, *Hyla caerulea* selective distribution of blood occurs under certain unknown circumstances while at others mixing of blood takes place. Thus experimental data are still inconclusive regarding the nature of the intracardiac circulation of blood in the amphibian and reptilian hearts. But looking at the problem from a purely anatomical point of view, one feels that several advanced features of the reptilian heart such as the absence of a truncus arteriosus; increased ridge formation towards the left side of the ventricle and relative lack of the same towards the right side; the presence of an incomplete interventricular septum whose incompleteness is such that the venous blood is obviously led on to the cavum pulmonale; all these and many more to be detailed presently, indicate an attempt at a selective distribution of blood within the reptilian ventricle. The degree of separation may, however, vary with different reptiles, depending upon the finer details of their cardiac anatomy.

The mesial auriculo-ventricular valves extend somewhat deeply into the cavum dorsale in the hearts of Lacertilia and Ophidia (Text-figs. 5-6). They are attached to the ventricular wall dorsally and ventrally and are free only laterally. Therefore, it could be expected that the arterial and venous streams of blood rushing into the ventricle push the lateral free margin of the valves, medially, as far as their fixed dorsal and ventral margins permit. By this the two mesial valves form a sort of a transient median vertical partition which is operative till the main volume of arterial and venous blood have found their respective places within the ventricle. Secondly, the incomplete interventricular septum is so situated that it permits the bulk of the venous blood to get into the cavum pulmonale from where it is carried forward by the pulmonary trunk. Towards the left side of the cavum dorsale, the absence of an exclusive arterial chamber is amply compensated by the numerous vertical crevices that cut deep into the wall of the ventricle. These locked-up spaces are decidedly more advantageous than open cavities as they do not permit a free movement of the blood lodged in them and hence prevent, to some extent, the mixing up of the two streams of blood.

The location of the openings of the three aortic trunks is also noteworthy. The opening of the pulmonary trunk is within the cavum pulmonale, ventral to the interventricular septum. The opening of the right systemic trunk is dorsal to the septum, very near the right auriculo-ventricular aperture in such a way that it is just covered over by the lateral margin of the right auriculo-ventricular aperture during the ventricular systole. The opening of the left systemic trunk lies just beyond the lateral margin of the right auriculo-ventricular valve when the latter is distended, opposite to the free margin of the inter-ventricular septum. The disposition of these openings is very suggestive of a selective distribution of blood.

It has been suggested that the ventricle of the lower reptile has within it two incomplete septa disposed in different planes. One of these is the interventricular

septum. The other one has been referred to as the "muscular ridge" by Rau (1924) and "vertical septum" by Leene and Vorstman (1930). The vertical septum is said to be situated about the middle of the cavum dorsale, in its caudal portion, disposed in a dorso-ventral plane. The vertical septum, by virtue of its location, appeared to them as significant and suggestive of the way in which the interventricular septum may have become complete. A similar opinion is implied when Foxon (1955) says that "it is this secondary septum which in crocodiles and birds become the definitive interventricular septum". The only thing that was required was to continue this incomplete, caudal and vertical septum, cranially, till it joins the region between the mesial valves of the auriculo-ventricular apertures. Before becoming complete, the vertical septum should, however, turn to the right so as to include the opening of the right systemic trunk. Leene and Vorstman (1930) summed up their hypothesis by saying that "in dividing the ventricle into two parts, two septa play a part, a vertical septum and a horizontal one". The horizontal septum referred to here is the interventricular septum. They also illustrated their hypothesis by means of two figures in which the vertical and the horizontal septa have been extended further, anteriorly, along the planes they occupy, till the ventricular cavity is completely divided into venous and arterial channels.

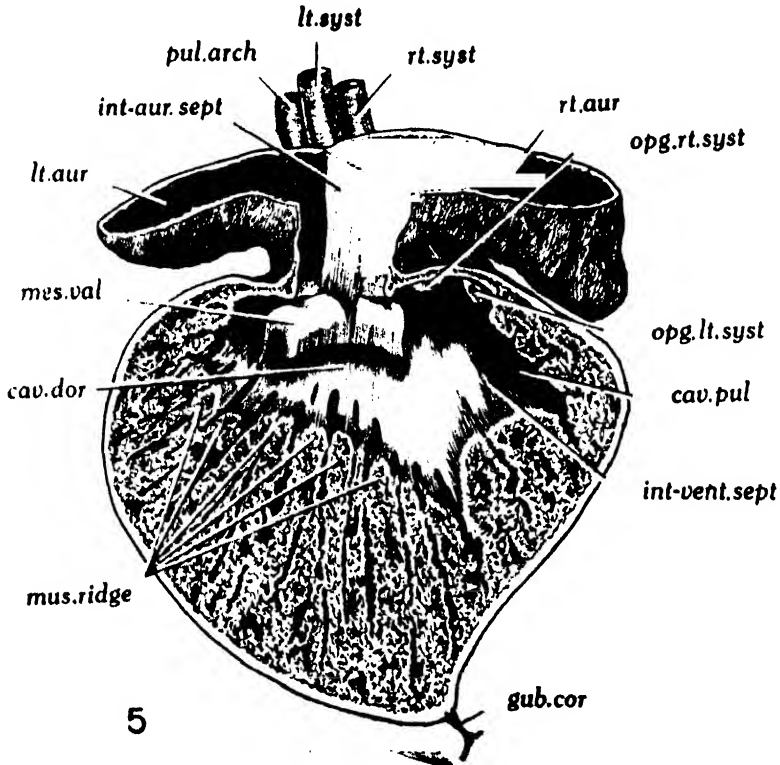


TEXT-FIG. 4.

Dissection of the ventricle of *Geomyda trijuga* from the dorsal side, showing the auricular ostia with valves, the cavum dorsale, the openings of the aortic trunks and the incomplete interventricular septum. Note the spacious cavum dorsale and the moderately thick wall of the ventricle. The small ridge between the openings of the two systemic trunks indicates the position occupied by the pars endocardialis in the crocodilian ventricle.

The present study has afforded an opportunity to find out the validity of Leene and Vorstman's two-septa hypothesis. Transverse sections of the ventricle of many reptiles, particularly of *Lacertilia* and *Ophidia* show a medially situated

and dorso-ventrally disposed muscular column which would answer to the descriptions of a vertical septum. It is situated in such a way as to divide the cavum dorsale into left and right halves, if extended anteriorly. Transverse sections, however, do not give the required topographical details which would indicate the real nature of this vertical septum. When the ventricle is dissected, it is often difficult to locate the vertical septum from among the numerous vertical ridges that project into the cavum dorsale from behind. In reality, the so-called vertical septum appears to be the most medial and prominent of these vertical ridges and nothing more. In *Chelonia* (Text-fig. 4), where the wall of the ventricle, which is responsible for these vertical ridges, is relatively thin these ridges do not extend far into the cavum dorsale and this is probably the reason why Leene and Vorstman (1930) were not able to find a well developed vertical septum in *Chelone*. In *Testudo* they reported that the vertical septum is much better developed and this may be due to a slight increase in the muscularity of the ventricular wall. Thus the vertical septum which is to be entrusted with so important a role as the completion of the interventricular septum is found to be very inconstant in its structure and position and is often poorly developed too.



Dissection of the ventricle of *Calotes versicolor* from the dorsal side, showing the internal structure. Note the increase in thickness of the wall of the ventricle with a corresponding reduction in the extent of the cavum dorsale. The muscular ridges conceal deep crevices among them.

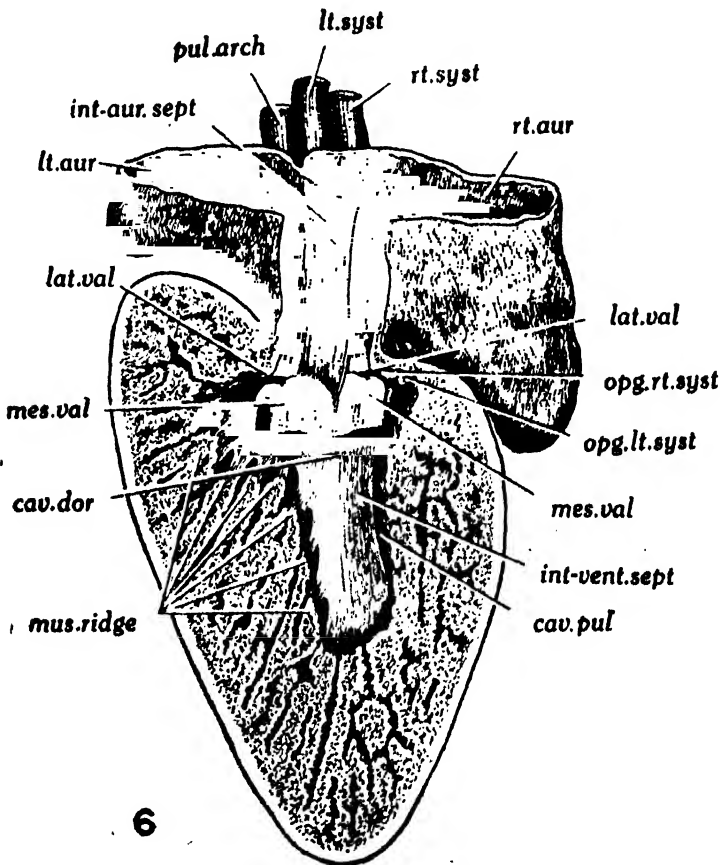
Besides, there is the problem of the opening of the right systemic trunk which has to be brought towards the left side of the ventricle before the vertical septum completes itself anteriorly. This problem is explained away by Leene and Vorstman

(1930) by assuming that "the vertical septum turns more or less to the right growing in a cranial direction". It is difficult to understand how the vertical septum, which is already within easy reach of the region between the two mesial valves, could turn to the right and include the opening of the right systemic trunk and at the same time leave out the right auriculo-ventricular aperture.

It is also worth noting that most of the valves and septa are endocardial in origin and often remain in the same condition throughout, while the vertical septum is purely muscular except for an extremely thin covering of endocardial tissue in common with the lining of the cavum dorsale.

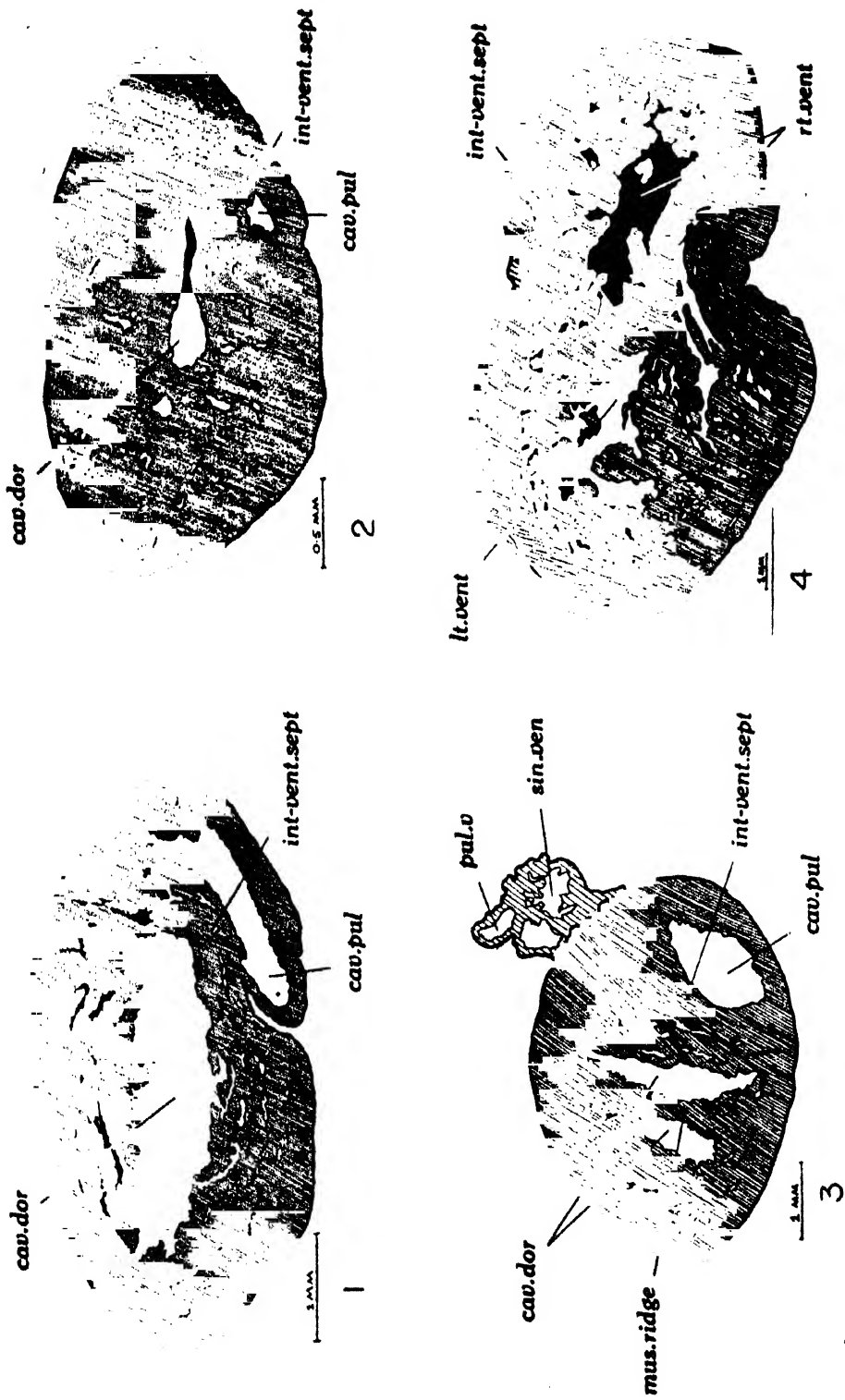
Further, it is difficult to believe that the completion of the interventricular septum is effected from the caudal end as Goodrich (1919) has opined, so far away from the region of the auricular ostia and the opening of the systemic trunks with which the finished septum is so intimately associated.

Thus from the above account it becomes clear that the vertical septum of the lower reptiles cannot possibly effect the completion of the interventricular septum. As shall be detailed later, the problem is intimately bound up with the incomplete interventricular septum of the lower reptiles.



TEXT-FIG. 6.

Dissection of the ventricle of *Naja naja* from the dorsal side, showing the internal structure. Note the thickness of the wall of the ventricle and the mesial shift of the interventricular septum.



TEXT-FIG. 7.

Transverse sections of the ventricle through the sub-apical region in the hearts of (1) *Geomyda trijuga*, (2) *Calotes versicolor*, (3) *Naja naja* and (4) *Crocodilus palustris*. The interventricular septum is complete and separates the large cavum dorsale from the small cavum pulmonale in 1, 2 and 3 and separates the right and left ventricles in 4.

The interventricular septum or the horizontal septum is the most important component of the ventricle and exhibits a remarkable constancy of structure, position and function in the heart of lower reptiles. It consists of two parts, a posterior complete (Text-fig. 7, figs. 1-3) and an anterior incomplete part (Text-fig. 7, figs. 5-7). The posterior portion is made up of a loose assemblage of muscle fibres and is recognised by its characteristic oblique position and the presence of the *cavum pulmonale* towards its right. The anterior portion is very clearly differentiated because of its smooth endocardial covering and its characteristic horizontal position. It forms the floor of the *cavum dorsale* towards the right side and its free margin overhangs the *cavum pulmonale*. In *Chelonia*, the horizontal septum is rather short and is situated diagonally towards the right anterior corner of the ventricle (Text-fig. 4). In *Lacertilia*, it is somewhat longer and more mesial than in *Chelonia* and therefore, the *cavum pulmonale* which lies towards its right appears to be more extensive (Text-fig. 5). The lengthening of the horizontal septum and its mesial shift is further emphasised in the Ophidian ventricle in which the free margin of the horizontal septum is brought almost in a line with the right auriculo-ventricular aperture (Text-fig. 6). This enables the *cavum pulmonale* to receive almost all of the venous blood as it rushes into the ventricle, thereby minimising the chances of its mixing with the arterial blood.

The free margin of the horizontal septum, particularly at its anterior end, has a thick covering of endocardial tissue which is continuous with that of the pocket valves of the aortic trunks.

Regarding the disposition of the openings of the aortic trunks Goodrich (1930) very aptly says that "the *cavum arteriosum* leads, antero-dorsally, to the septum towards the opening of the right carotico-systemic trunk, while the opening of the left systemic trunk is situated almost opposite the free edge of the septum. . . The position of the opening into the left systemic trunk varies a little in different forms, being nearer the opening of the right trunk in *Ophidia*, and of the pulmonary trunk in *Chelonia*, but the general disposition of the three openings is remarkably constant throughout *Reptilia*". The nearness of the opening of the left systemic trunk to the opening of one or the other of the remaining two aortic trunks has been the basis for attempts at dividing the lower reptiles into two groups, with some phylogenetic significance attached to such a division (O'Donoghue, 1918; von Hofsten, (1941). In reality, the nearness of the opening of the left systemic trunk to the opening of either the right systemic or the pulmonary trunk cannot form a clue to the actual position of the incomplete interventricular septum.

In the present study an attempt is made to mobilise all the indirect evidence which would establish the true position of the interventricular septum with reference to the opening of the aortic trunks. This shows that the interventricular septum of lower reptiles lies between the left systemic and pulmonary trunks, as figured for the *Lepidosaurian* type by von Hofsten (1941).

(1) In dissections of the ventricle in which the dorsal wall has been removed, the openings of both the systemic trunks are seen lying side by side, with a common wall between them, dorsal to the free margin of the interventricular septum. Only the ventrally situated opening of the pulmonary trunk is hidden from view.

(2) There is invariably a confluent space posterior to the openings of the two systemic trunks and their pocket valves are made up of the same continuous endocardial tissue. This fact is clearly seen in transverse sections of all the hearts studied (Text-fig. 9-11).

(3) On the other hand, transverse sections show clearly that the pulmonary trunk is completely separated from the two systemic trunks by a muscular wall. There is not a single instance of such an encroachment of muscular wall between the two systemic trunks, within the limits of the ventricle. It should be noted that

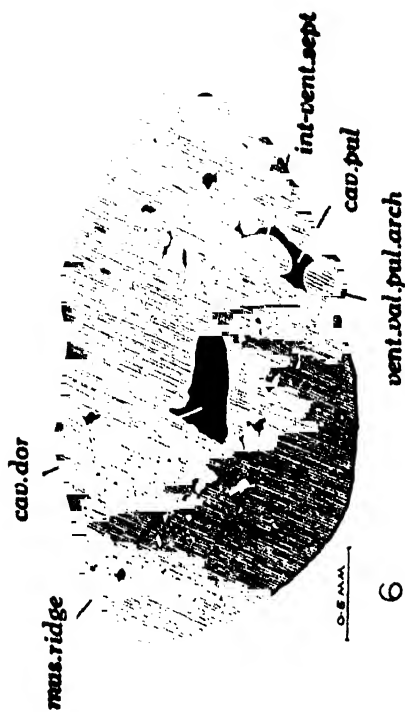
the muscular elements that are seen between the pulmonary and systemic trunks become continuous with the free margin of the interventricular septum, posteriorly.

(4) It is an accepted fact that a part of the bulbus arteriosus of the anamniote heart becomes incorporated into the reptilian ventricle during the latter's phylogeny and contributes to its definitive right wall (Greil, 1903; O'Donoghue, 1912; Robertson, 1914; Goodrich, 1930). For this reason, during the development of the reptiles, the posterior end of the spiral fold or its homologue and the anterior end of the interventricular septum meet at the ventral endocardial ridge of the auriculoventricular aperture and become continuous with each other. As the spiral fold is situated between the arterial and venous channels, it effects the complete separation of the pulmonary trunk from the two systemic trunks during the development of the reptilian heart (Bremer, 1928; Goodrich, 1930). As the spiral fold is continuous with the anterior end of the interventricular septum it can be inferred that the real position of the latter is between the pulmonary and the systemic trunks.

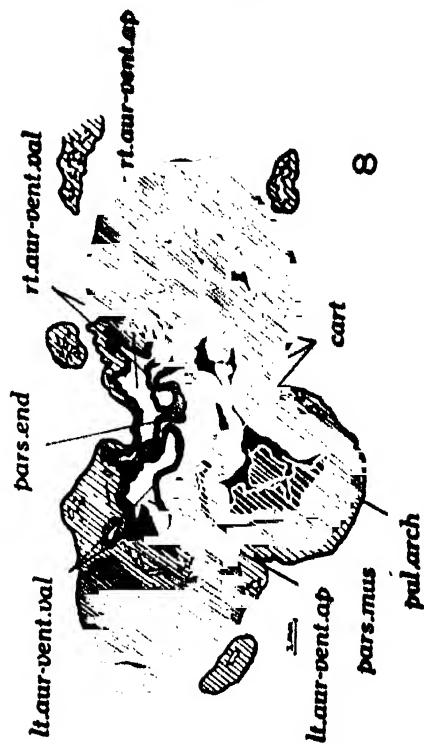
(5) Another convincing proof that the opening of the left systemic trunk is definitely dorsal to the interventricular septum is the cartilaginous support. In those hearts in which there is a cartilaginous support, it is always situated at the free margin of the interventricular septum and extends, to a greater or lesser extent, between the pulmonary and the left systemic trunks.

Therefore, O'Donoghue's contention that the living reptiles can be divided into two groups regarding the position of the interventricular septum with reference to the opening of the aortic trunks is untenable. It should, however, be pointed out that the conclusion arrived at in this study refers to the condition seen in the living lower reptiles and not to the course phylogeny might have taken in the completion of the interventricular septum.

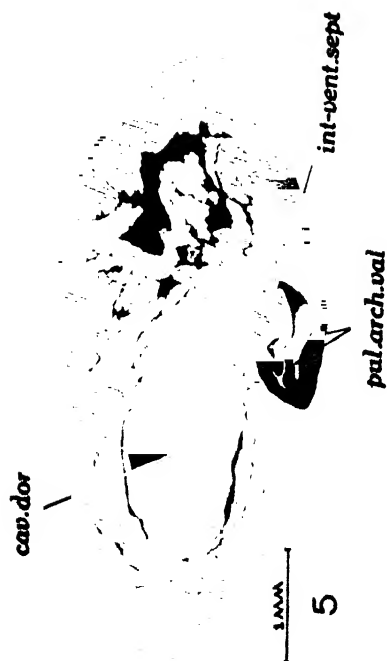
A careful comparison of the structure and position of the complete interventricular septum of the crocodile with the incomplete interventricular septum of lower reptiles, gives a clue to the manner in which it may have become complete. The crocodilian interventricular septum consists of two parts, an antero-dorsal and a postero-ventral (Text-fig. 7, fig. 4; Text-fig. 8, fig. 8; Text-fig. 9, fig. 12). The two parts are sharply marked off from one another in their structure and position although, they form a single continuous septum. The postero-ventral portion is very thick and muscular and is called *pars muscularis*. It bears a striking resemblance to the incomplete interventricular septum of lower reptiles. In transverse sections of the ventricle the appearance of the two is exactly the same, both regarding the bend towards the right side and the position of the openings of the aortic trunks. Instead of having a free margin, as in lower reptiles, the *pars muscularis* is continued dorsally by the antero-dorsal portion of the interventricular septum, *pars endocardialis*. If the *pars endocardialis* is removed from the crocodilian ventricle its internal structure would be very similar to that of the ventricle of a lizard or snake. In accordance with this interpretation, the *pars endocardialis* and the *pars muscularis* should meet at an angle along a line which corresponds to the free margin of the interventricular septum of lower reptiles. Dissections as well as transverse sections of the crocodilian ventricle show that this is precisely the case and the fold formed at the junction of the dorsal and ventral portions of the interventricular septum is clearly seen in them. From the above account it becomes evident that the right ventricle of the crocodilian heart is merely the *cavum pulmonale* of the lower reptile with parts of the *cavum dorsale* about the free margin of the interventricular septum added to it. The *pars endocardialis* of the crocodilian ventricle has no counter-part within the ventricle of lower reptiles. It is not homologous to the so-called vertical septum because the latter is merely one of the numerous ridges present towards the left side of the ventricle and is very inconstant in structure and position. Further, the vertical septum joins the interventricular septum, ventrally, at the



6



8



5



7

TEXT-FIG. 8.

Transverse sections of the ventricle through the middle region in the hearts of (5) *Geomys trijuga*, (6) *Calotes versicolor*, (7) *Naja naja* and (8) *Crocodilus palustris*. The interventricular septum is incomplete and horizontal in 5, 6 and 7, and is complete in 8. Note that in 8, the interventricular septum consists of two parts, a dorsal thin pars endocardialis and a ventral thick pars muscularis. The latter bears cartilaginous nodules.

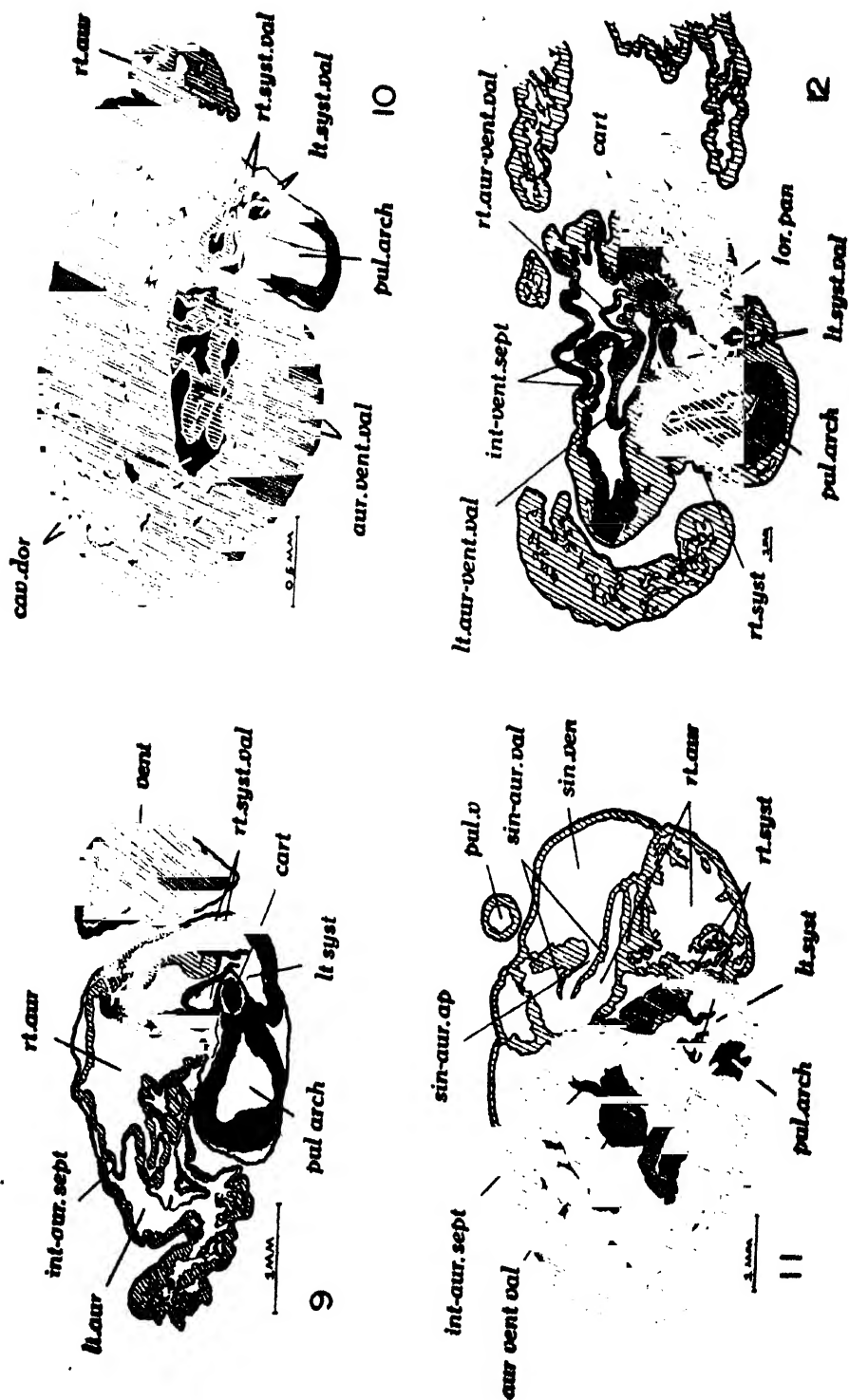
base of the latter, while the pars endocardialis joins the pars muscularis along a line which corresponds to the free margin of the incomplete interventricular septum. The pars endocardialis is, therefore, a new formation.

It is known that there are four endocardial ridges in the embryonic bulbus of reptiles (Text-fig. 10 fig. 16). These are termed the ventral, dorsal, left and right ridges (Bremer, 1928; Goodrich, 1930). Of these, the ventral one is the largest and corresponds to the amphibian spiral fold. The fusion across of the ventral and dorsal ridges results in the formation of the septum pulmo-aorticum which separates the pulmonary arch from the two systemic trunks. The fusion across of the right and left ridges results in the formation of the septum aorticum which separates the right and left systemic trunks. The right endocardial ridge is, however, rudimentary and hence the pulmonary channel remains a single undivided arch in the region of the bulbus. As already mentioned, the interventricular septum is in continuity with the ventral endocardial ridge. On analogy, it could be suggested that the pars endocardialis is in a line with the left ridge that separates the two systemic trunks and thus forms its ventricular extension. If such a condition is drawn, the diagram bears a striking resemblance to the cross section of a crocodilian ventricle in all details (Text-fig. 10, fig. 16). The dorsal ridge is not represented in this hypothetical cross section because it restricts itself to the region of the bulbus and does not extend into the ventricle in any reptile. The right ridge is also not to be represented because, as already pointed out, it is rudimentary even in the region of the bulbus.

In the formation of the complete interventricular septum, therefore, the endocardial elements play an important part as is normally the case (Robertson, 1913; Davis, 1927). The thick covering of endocardial tissue at the anterior end of the incomplete interventricular septum makes this hypothesis probable. Further, in *Geomyda trijuga* the anterior end of the interventricular septum gives off a small endocardial ridge which passes between the openings of the two systemic trunks (Text-fig. 4). This is exactly the position of the pars endocardialis in the crocodilian ventricle.

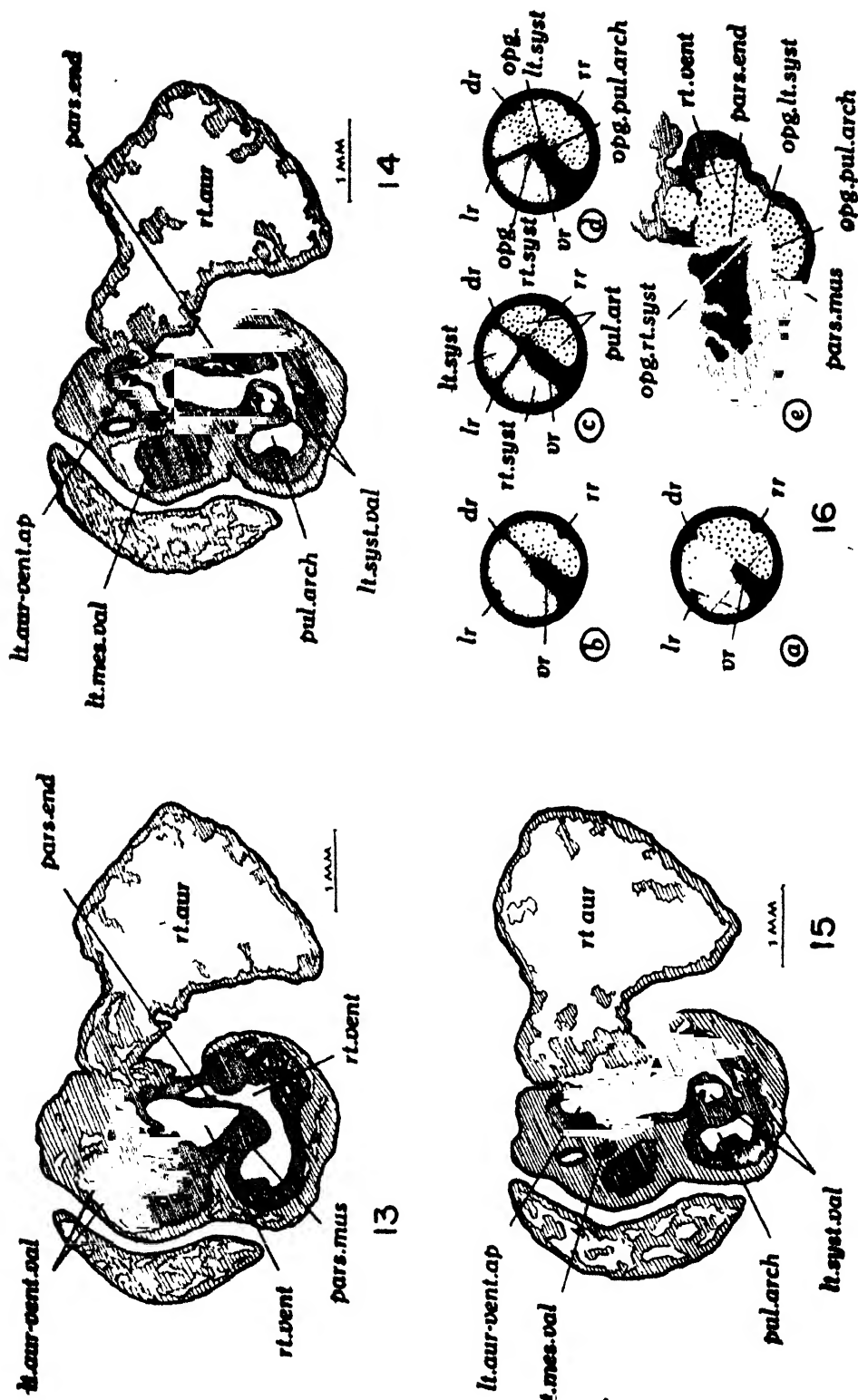
It will also be seen that by such a hypothesis the openings of the three aortic trunks are automatically set in the position they occupy in the crocodilian ventricle, without involving the turning and twisting of any structure that is already present in the ventricle of living reptiles. In order to bring the region of the auricular ostia in a line with the two systemic trunks, as seen in the crocodilian heart, one other change is necessary in the ventricle of a lower reptile. The interventricular septum with the openings of the aortic trunks at its anterior end should move towards the middle of the ventricle at a plane ventral to that of the auriculo-ventricular apertures. It has already been pointed out that such a shift is seen, to a slight degree, in the lacertilian and, to a marked degree, in the ophidian ventricles. This suggests the way by which a mesial shift of the interventricular septum may have taken place in the evolution of the crocodilian condition and to that extent the mesial shift recorded in the lacertilian and ophidian hearts is a proof to the hypothesis that has been put forth.

Another proof for the hypothesis comes from a developmental anomaly found in the heart of a crocodile embryo. Transverse sections of the heart of this embryo crocodile, which was fully developed and measured 10 cms., showed that the interventricular septum is incomplete anteriorly (Text-fig. 10, figs. 13-15). About the level of the mesial valve of the left systemic trunk, the pars endocardialis is found to be dissociated from the pars muscularis, just where they meet at an angle. Anterior to this point, the pars endocardialis is seen to gradually dwindle away from the pars muscularis and finally disappear altogether. That this is not an artefact is evident by the fact that after its dissociation from pars muscularis, the pars endocardialis could be followed up serially in a number of sections, each section showing



TEXT-FIG. 9.

Transverse sections of the ventricle through the aortic trunks in the hearts of (9) *Geomys triinga*, (10) *Calotes versicolor*, (11) *Naja naja* and (12) *Crocodilus palustris*. Note that in 9, 10 and 11 the right and left systemics are confluent with one another, and cut off from the pulmonary trunk by a muscular wall. A similar situation is seen in *Crocodilus palustris* in the region of the foramen of Panizzae.



TEXT-FIG. 10.

Figures 13, 14 and 15.—Transverse sections of the ventricle of *Crocodilus palustris* showing an anomalous incompleteness in the interventricular septum. Figure 16 is a comparison between the embryonic bulbus of reptiles with the crocodilian ventricle. Explanation in the text.

it slightly shorter than it is in the previous one, till it completely disappears in the anterior sections. This anomaly points out to the fundamental duality of the crocodilian interventricular septum, a new endocardial septum becoming associated, in the course of phylongey, with a pars muscularis already existing in the ventricle of lower reptiles in the form of an incomplete interventricular septum.

Summary

The present study, based on the hearts of 29 species of reptiles, has brought to light the following facts concerning the structure of the reptilian heart.

1. The shape of the heart bears a direct relation to the external form of the body. In forms in which the shape of the body is in any way modified, the shape of the heart is also seen to be altered.

2. An auricular diverticulum is commonly seen in the hearts of lizards. It is usually borne on the antero-mesial aspect of the right auricle.

3. The junction of the left precaval vein with the sinus venosus is constricted in some lizards and most snakes. The presence of such a constriction is associated with a well developed sinus septum within the sinus venosus.

4. The sinu-auricular aperture is elliptical and placed obliquely transverse. It is bound by well developed cephalic and caudal valves.

5. The ventricular wall is particularly thick towards the left side and is beset with numerous vertical ridges and spacious crevices. These crevices trap the arterial blood as it enters the ventricle and hold it till the main current of venous blood reaches the cavum pulmonale.

6. The vertical septum which is supposed to divide the cavum dorsale into right and left halves is found to be inconstant in structure and position. It is merely one of the many vertical ridges found in the wall of the ventricle towards the left side. It is unlikely that it plays any significant role in the completion of the interventricular septum.

7. The incomplete interventricular septum of lower reptiles shows a remarkable uniformity of structure. It is complete and vertical posteriorly and incomplete and horizontal anteriorly.

8. It has been found that, in all the lower reptiles, the anterior end of the incomplete interventricular septum lies between the pulmonary and systemic trunks and the division of the lower reptiles into two groups on the basis of the position of the interventricular septum is, therefore, untenable.

9. Hearts of Chelonia, Lacertilia and Ophidia form a progressive series showing a gradual increase in the thickness of the ventricular wall towards the left side and a shifting of the interventricular septum towards the middle of the ventricle, indicating the line of evolution the ancestors of the crocodiles may have adopted to effect the complete division of their ventricle.

10. Pars muscularis of the crocodilian interventricular septum is homologous with the incomplete interventricular septum of lower reptiles. Pars endocardialis is a new formation without any corresponding structure within the ventricle of living lower reptiles. It is, presumably, an elaboration of the endocardial covering of the free margin of the incomplete interventricular septum, in a line with the left endocardial ridge of the embryonic bulbus.

ACKNOWLEDGEMENTS

The author wishes to record his gratitude to Prof. P. W. Gideon, Head of the Department of Zoology, Karnatak College, Dharwar, at the time when this study was undertaken, for his kindly encouragement and helpful suggestions. The author also considers himself fortunate in having the unstinted co-operation and expert guidance from Dr. K. R. Karandikar who succeeded Prof. P. W. Gideon as the Head of the Zoology Department, Karnatak College, Dharwar.

LIST OF ABBREVIATIONS

aur. vent. val. : auriculo-ventricular valve.
cart. : castilago.
caud. val. : caudal valve of the sinu-auricular aperture.
cav. dor. : cavum dorsale.
cav. pul. : cavum pulmonale.
ceph. rim. : cephalic of the sinu-auricular aperture.
ceph. val. : cephalic valve of the sinu-auricular aperture.
com. car. : common carotid trunk.
divert. : diverticulum.
dr. : dorsal endocardial ridge.
for. pan. : foramen of Panizza.
gub. cord. : gubernaculum cordis.
int. aur. fis. : interauricular fissure.
int. aur. sept. : interauricular septum.
int. vent. sept. : interventricular septum.
lr. : left endocardial ridge.
lat. val. : lateral valve of the auriculo-ventricular aperture.
lt. aur. : left auricle.
lt. aur. vent. apert. : left auriculo-ventricular aperture.
lt. car. : left carotid artery.
lt. mes. val. : left mesial valve of the auriculo-ventricular aperture.
lt. precav. : left precaval vein.
lt. pul. v. : left pulmonary vein.
lt. syst. : left systemic trunk.
lt. syst. val. : valve of the left systemic trunk.
lt. vent. : left ventricle.
mes. val. : mesial valve of the auriculo-ventricular aperture.
mus. ridge. : muscular ridge.
opg. lt. syst. : opening of the left systemic trunk.
opg. pul. arch. : opening of the pulmonary arch.
opg. rt. syst. : opening of the right systemic trunk.
pars. end. : pars endocardialis.
pars. mus. : pars muscularis.
postcav. : postcaval vein.
pul. arch. : pulmonary arch.
pul. arch. val. : valve of the pulmonary arch.
pul. art. : pulmonary artery.
pul. v. : pulmonary vein.
rt. aur. : right auricle.
rt. aur. vent. apert. : right auriculo-ventricular aperture.
rt. aur. vent. val. : right auriculo-ventricular valve.
rt. car. : right carotid artery.
rt. precav. : right precaval vein.
rt. pul. v. : right pulmonary vein.
rr. : right endocardial ridge.
rt. syst. : right systemic trunk.
rt. syst. val. : valve of the right systemic trunk.
rt. vent. : right ventricle.
sin. aur. apert. : sinu-auricular aperture.
sin. aur. val. : sinu-auricular valve.
sin. sept. : sinus septum.
sin. ven. : sinus venosus.
vent. : ventricle.
vr. : ventral endocardial ridge.
vent. val. pul. arch. : ventral valve of the pulmonar arch.

LIST OF REPTILES STUDIED

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|------------|--|
| Chelonia | 1. <i>Geomyda trijuga</i> , Schweigger (Emydidae). |
| | 2. <i>Testudo elegans</i> , Schoepff (Testudinidae). |
| | 3. <i>Lissemys punctata</i> , Bonaterre (Trionychidae). |
| Lacertilia | 4. <i>Hemidactylus leschenaulti</i> , Dum and Bibr (Gekkonidae). |
| | 5. <i>Teratolepis fasciata</i> , Blyth (Gekkonidae). |
| | 6. <i>Calotes versicolor</i> , Daudin (Agamidae). |
| | 7. <i>Chamaeleon zeylanicus</i> , Laurenti (Chamaeleonidae). |
| | 8. <i>Mabuya carinata</i> , Schneider (Scincidae). |
| | 9. <i>Riopa guentheri</i> , Peters (Scincidae). |
| | 10. <i>Barkudia insularis</i> , Annandale (Scincidae). |
| | 11. <i>Ophisops beddomei</i> , Jerdon (Lacertidae). |
| | 12. <i>Varanus monitor</i> , Linn (Varanidae). |
| Ophidia | 13. <i>Typhlops acutus</i> , Dum and Bibr (Typhlopidae). |
| | 14. <i>Typhlops braminus</i> , Daudin (Typhlopidae). |
| | 15. <i>Uropeltis phipsoni</i> , Mason (Uropeltidae). |
| | 16. <i>Python molurus</i> , Linn. (Boidae). |
| | 17. <i>Eryx johnei</i> , Russel (Boidae). |
| | 18. <i>Acrochordus granulatus</i> , Schneider (Colubridae). |
| | 19. <i>Oligodon taeniolatus</i> , Jerdon (Colubridae). |
| | 20. <i>Lycodon striatus</i> , Shaw (Colubridae). |
| | 21. <i>Dryocalamus nympha</i> , Daudin (Colubridae). |
| | 22. <i>Balanophis ceylonensis</i> , Gunther (Colubridae). |
| | 23. <i>Macropisthodon plumbicolor</i> , Cantor (Colubridae). |
| | 24. <i>Boiga trigonata</i> , Schneider (Colubridae). |
| | 25. <i>Dryophis pulverulentus</i> , Dum and Bibr (Colubridae). |
| | 26. <i>Naja naja</i> , Linn. (Elapidae). |
| | 27. <i>Vipera russelli</i> , Shaw (Viperidae). |
| | 28. <i>Trimeresaurus gramineus</i> , Shaw (Viperidae). |
| Crocodylia | 29. <i>Crocodylus palustris</i> , Lesson (Crocodylidae). |

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THYROID AND MALE FERTILITY IN FARM ANIMALS*†

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ABSTRACT

The article deals with the effect of feeding thyroactive materials on the reaction time and semen characteristics of goats and buffaloes during the part of the year when the semen quality of these two species of farm animals is relatively poor. In goats it has been found that feeding of thyroprotein (Protamone) at a level of 1 gm./animal/day, over a period of 3 months (May to August) had no significant effect on the reaction time, initial motility of spermatozoa, total number of spermatozoa per ejaculate, methylene blue reduction time of semen and percentage of abnormal spermatozoa. A highly significant decrease in semen volume was observed due to the administration of the drug. Sperm concentration per unit volume of semen increased due to the feeding of the drug with concomitant decrease in initial fructose content of semen and increase in the rate of fructolysis by spermatozoa.

In buffalo bulls, it has been observed that feeding of sodium-L-thyroxine at a rate of 100 mg./animal/day for a period of 14 weeks (August to November) did not have any effect on reaction time and sperm concentration. The administration of the drug, however, decreased the semen volume, initial fructose content of semen and the percentage of abnormal spermatozoa and increased the initial motility of spermatozoa and fructolysis index. The effect of the drug on the seminal characteristics, except the fructolysis index, continued during the observed period of 13 weeks after the administration of the drug was stopped.

Thyroid gland plays a significant role on fertility of animals including the farm animals, though opinion on the nature and pathways of action of the thyroid hormones on reproduction varies. A number of investigators hold the view that the thyroid has no direct influence on reproduction. Any reproductive disturbance in the male or female in hypo or hyperthyroidism is primarily due to changed metabolic status (Moore, 1939, Anderson, 1948), changed nervous irritability (Lerman, 1942), general growth disturbance (Schneider, 1939) or to the complex interrelations between the endocrine system and body metabolism as a whole (Cameron, 1945) rather than to specific endocrine imbalance.

Thyroidectomy in the bull resulting in complete clinical myxoedema leads to a complete disappearance of libido and loss of interest in the estrual female (Petersen *et al.* 1941). Spermatogenesis is not inhibited, for ejaculates from such bull obtained by massage of the ampulla are normal in sperm activity, morphology, longevity and fertilizing ability. These investigators also brought about a complete restoration of normal libido and sexual behaviour in bull by the administration of the thyroid or testosterone propionate. A metabolic stimulant dinitrophenol produces similar effect as do desiccated thyroid or methyl testosterone (Petersen *et al.* 1941). This suggests that the effect is largely one of lowered general metabolism rather than a specific endocrine one. Reineke (1946) found definite improvement in ten out of fourteen aged bulls (average age 8 years). Definite evidence of improvement of the conception record was obtained in only four cases. The results of this experiment further support the above contention, for it is well established that metabolism declines with advancing age (Brody, 1945). Schultze and Davis (1946) observed increased conception rate, higher sperm motility and greater resistance of spermatozoa to low temperature (4°), storage in five out of seven iodinated casein fed bulls. The proportion of abnormal sperm did not change. The improved conception rate remained during the post treatment period.

* In this paper investigations conducted by different workers at the I.V.R.I. are presented. The names of the investigators have been indicated with each experiment.

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Sahiwal bulls are very slow at service and Zebu bulls in Kenya a little better (Anderson 1956). Thyroid therapy has been successfully applied by Anderson (1944b) in sluggish bulls.

Experiments with laboratory animals indicate that the retardation in sexual function that is observed following thyroidectomy is due at least in part to a decline in the gonadotrophic hormones of the hypophysis. Reduced and increased response to gonadotrophic hormone in female mice by feeding thiouracil and optimal levels of iodinated casein respectively (Meites and Chandrashekhar 1948) indicate a further thyroid-pituitary-gonadal relationship. The directly opposite results obtained in rats undoubtedly point to different hormone balances in the two species. Bioassays of the pituitary gland of thyroidectomized goats (Reineke *et al.* 1941), and rabbits (Chu 1944) showed a marked decrease in gonadotrophic hormone content when compared with assays of normal control glands.

Thyroid hormone (Thyroxine) also exerts direct influence on the metabolism of bovine semen (Schultze and Davis, 1947, 1948). Thyroxine added to bull semen in a critical concentration range brought about an increase in oxygen uptake of samples with sperm concentration above 8 millions per ml. But semen samples of lower concentration were not affected. These workers also reported increased conception rate by direct addition of thyroxine in bovine semen.

Berliner and Warbritton (1937) noted that thyroidectomy in rams resulted in a reduction in sperm number and an increase in the percentage of abnormal spermatozoa, edematous tests, decrease in the interstitial tissues, sloughing and pycnotic seminiferous tubules. The fall in sperm production was restored by thyroxine injection. Bogart and Mayer (1946) observed a marked reduction in sperm number and decline in motility and an increase in the proportion of abnormal sperm in thiouracil fed hypothyroid rams. Though no specific observation on libido was made during collection of semen in artificial vagina, sex interest appeared to be impaired during the experiment period of 40-50 days. The thiouracil effect was reversed by feeding iodinated casein. Turner *et al.* (1943) found that a Toggenburg buck which showed a definite lack of interest in oestrous does, displayed sexual interest after the feeding of 1 g. thyroprotein per day.

It appears probable from the results of various investigators that the thyroid exerts its influence on reproduction by way of one or more of several mechanisms (Reineke 1946).

- (1) by the general calorogenic effect on all cells, including those of the endocrine system,
- (2) by a specific effect on the gonadal cells or
- (3) by a specific stimulating influence on the pituitary gland.

The thyroid gland also seems to play a significant part in the established relationship between seasonal variations in climatic conditions and reproductive functions in farm animals (Anderson 1956). The question of the influence of thyroid status on male reproduction in farm animals assumed practical importance in view of the temporary "summer infertility" of rams during the hot weather (McKenzie and Berliner, 1937) which has many common features with hypothyroidism. Some of the symptoms of summer infertility excepting low sperm motility and low semen volume are alleviated by thyroxine administration. Bogart and Mayer (1946) in confirmation of the earlier findings of Berliner and Warbritton (1937) demonstrated that "hyper thyroidism in the normal ram increased sperm production and decreased abnormal sperm during the summer months" (Blaxter *et al.* 1949). A season of increased temperature and sunshine is associated with increased libido, higher density and motility of sperm, better maintenance of useful motility, lower pH of semen, better fertility in the moderate climate of Kenya. (Anderson, 1944a, 1945). During the period July to August when semen quality in bulls is relatively poorer, Anderson (1956) could not find any improvement of libido which was initially good, or of sperm density or of pH change

on incubation, but there was a highly significant individual improvement of motility by thyroid therapy. But other bulls giving semen of poor motility and high initial pH during the adverse period improved in both respects after treatment. Bogart and Mayer (1946) postulated that the effect of season on reproduction is due to the widely known relation between environmental temperature and thyroid hormone secretion rate (Mills, 1918, Dempsey and Astwood, 1943) and that the thyroid gland is of direct importance in maintaining ram fertility. Summer infertility may also be due to the well known deleterious effect of high scrotal temperature on spermatogenesis (Glover 1956) or to a lowered metabolism of the rams in the summer when food intake goes down or to a combined effect of both. Warwick *et al.* (1948) reported that rams which tolerated the dosage of thyroprotein during summer produced semen either equal in quality or slightly better than that from control group. But Eaton *et al.* (1948) could not find any such improvement in ram's semen during summer by administering a higher dose of thyroprotein (1—2 g.).

The present study on the effect of thyroid substance on reproduction in male goats and buffalo bulls during the hot weather was necessitated by the absence of any information on the influence of thyroid therapy on male reproduction in Indian farm animals during the season of increased temperature, humidity and rainfall. During this season the semen quality of rams and goats (Shukla and Bhattacharya, 1952 *a* and *b*), of buffalo bulls (Kushwaha, Mukherjee and Bhattacharya, 1955) and the quality of semen and blood in bulls (Mukherjee and Bhattacharya, 1952 *a* and *b*) were adversely affected. The adverse effect of season on semen seems to be associated with decreased activities of the thyroid and testes in buffalo bulls (Bhatnagar, Mukherjee and Bhattacharya, 1955) and in rams and goats (Mukherjee, Joshi and Bhattacharya, 1959).

EXPERIMENT ON GOATS

I. Effect on Reaction Time and Semen Quality¹

Material and Methods

Fourteen male goats between the age of 1 to 2½ years were used in this experiment. Prior to the commencement of the experiment the animals were trained to give collection in an artificial vagina. The goats were distributed in four groups of seven each as evenly as possible on the basis of the following characteristics which were selected for judging semen quality. Throughout the experimental period the animals were kept under uniform dietary and managerial regime.

The animals of the treatment group were fed 1 gm. of thyroprotein (Protamone) per animal per day from the middle of May to middle of August. Semen was collected from each animal twice weekly by the same person and using the same anoestrous goat during the same hours of the day and was examined for

- (1) Volume
- (2) Initial motility
- (3) Sperm concentration per c.c.
- (4) Total number of spermatozoa per ejaculate
- (5) Percentage of abnormal spermatozoa

Reaction time was also recorded to estimate the libido or sex vigour by noting the time interval between the release of the goat near the she goat and the actual moment of ejaculation. During the treatment period all the above mentioned characteristics of semen were studied simultaneously. Initial motility was scored

¹ Experimenters : D. P. Mukherjee, A. Roy and P. Bhattacharya.

in terms of the criteria recommended by Erb *et al.* (1942). Sperm concentration was determined by haemocytometer method. Percentage of abnormal sperm was determined according to the method adopted by Mukherjee and Bhattacharya (1952a).

Table 1 shows the average reaction time and the average of the semen characteristics of the two groups, together with the results of statistical analysis of data regarding these characteristics during the entire experimental period.

It will be seen from the table that during the pre-treatment period, the two groups were almost identical in respect of the characteristics studied, except in the percentage of abnormal spermatozoa. The difference in the average percentage of abnormal spermatozoa between the two groups was found to be highly significant; the average in the experimental group being higher than in the control group.

During the treatment period, highly significant differences in volume of semen, sperm concentration and percentage of abnormal spermatozoa were found in the two groups. The differences in average reaction time and other attributes of semen as found between the two groups during the experimental period, were due to chance variation. The semen volume in the experimental group showed a highly significant decrease against that in the control group, but it showed a highly significant increase in sperm concentration. The difference in the percentage of abnormal spermatozoa remained highly significant during the treatment period, as it was during the pre-treatment period, the percentage in the experimental group was higher than in the control group. It seems, therefore, that the dosage of thyroprotein administered to animals had an adverse effect on semen volume. It undoubtedly increased the sperm concentration per ml. of semen, but failed to reduce the percentage of abnormal spermatozoa.

DISCUSSION

Semen Volume

The dosage of thyroprotein administered to the animals in the experiment was found to exert deleterious effect on semen volume which did not change during the pre and post treatment period. McKenize and Berliner (1937) observed that low semen volume which is a characteristic of hypothyroidism during summer infertility is not increased by thyroxine administration in such infertile rams. They could not find any reduction in volume due to thyroxine administration as in our experiment. Bogart and Mayer (1946) giving a dosage level of 1 gm. of thyroprotein and Black *et al.* (1950) did not report any adverse effect on semen volume of rams treated with thyroprotein. On the contrary, Eaton *et al.* (1948) observed increased semen volume in Shropshire and Hampshire rams fed 2 g. of thyroprotein per animal per day. They however, did not obtain the same result when the dose was reduced to 1 g.

Total sperm per ejaculate :

A consequential effect of such reduction in volume was that the total number of spermatozoa failed to show any significant difference between the two groups though there was a highly significant increase in sperm concentration per unit volume of semen of the treated group. Thus the present investigation shows that thyroprotein feeding has little effect on spermatogenesis which remained unaffected during the pre-treatment and treatment period.

Abnormal Spermatozoa :

The percentage of abnormal spermatozoa was not affected by feeding thyroprotein in this experiment. The present finding in this respect is not in agreement

TABLE I
Average of reaction time and semen qualities of control and experimental groups of goats

	Pre-treatment period			Treatment period				
	Control group	Experimental group	Difference between the two means	t value	Control group	Experimental group	Difference between the two	t value
Reaction time (in seconds)	31.3 ± 3.31	24.26 ± 1.60	7.04	1.786	32.6 ± 2.33	28.77 ± 1.06	3.9	1.429
Volume of seme in ml.	0.54 ± 0.04	0.43 ± 0.05	0.10	1.617	0.63 ± 0.03	0.50 ± 0.03	0.13	2.915**
Initial motility of spermatozoa	4.35 ± 0.11	4.16 ± 0.06	0.19	1.083	4.21 ± 0.04	4.29 ± 0.07	0.08	0.992
Sperm concentration in millions per ml. of semen	5368 ± 247.88	5025.00 ± 296.20	343	0.948	4323 ± 186.94	4987 ± 142.23	665	2.707**
Total number of spermatozoa in millions	2778 ± 242.0	2159 ± 222.0	619	1.855	2761 ± 21.99	2287 ± 128.6	474	1.769
Percentage of abnormal spermatozoa	3.35 ± 0.40	5.26 ± 0.56	1.91	2.852**	6.66 ± 0.30	9.62 ± 1.01	2.96	3.262

**Significant at one per cent.

****Significant at one per cent.**

either with that of Bogart and Mayer (1946) who found considerable decrease in abnormal spermatozoa by feeding thyroprotein for 30 days during summer at the same dosage level, or with that of Berliner and Warbritton (1937). The later group of investigators reported that two intact and one thyroidectomized Hampshire rams with a high proportion of abnormal forms returned to normal after the administration of two doses (2 mg.) of thyroxine injection in August. In contrast to these results Eaton *et al.* (1948) observed increased percentage of abnormal sperm after feeding thyroprotein to ram for 15 weeks. In the present experiment, some of the animals of the experimental group after 4 weeks of Protamone feeding produced semen containing up to 30 to 40 per cent beaded spermatozoa. They were included as abnormal sperm although such spermatozoa have been identified by Mukherjee and Bhattacharya (1949) as immature sperm. The increase in the percentage of beaded spermatozoa in some of the animals and their inclusion as abnormal sperm in the computation of results might have masked the beneficial effect of thyroprotein feeding on other true abnormalities. But in a study, from the point of view of semen utility, the beaded spermatozoa cannot be excluded from the computation of results because they, like the other abnormalities, reduce the fertilising capacity of semen.

Reaction time :

Sex vigour or libido as judged by reaction time does not appear to be adversely affected by conditions created by external factors such as high air temperature, relative humidity and rainfall under the conditions of the experiment. Therefore, administration of thyroprotein has little influence on sexual activity. There is practically no data on sheep and goat to compare this effect of thyroprotein on libido of goats in summer.

Initial motility :

Thyroprotein feeding did not influence initial motility and this finding is in agreement with that of Ahmed (1955) who could not find any marked change in the motility of spermatozoa of ram's semen. Warwick *et al.* (1948) found that semen from rams receiving 0.5 g. of thyroprotein had higher initial motility.

II. Effect on sperm nutrient and its utilization.¹

Apart from actual fertility tests, no one method is sufficient to assess the fertilizing capacity of semen. According to some investigators the rate of consumption of fructose, the only glycolisable sugar in semen has a positive correlation with fertility (Gassener, Hill, and Sultzburger, 1952). Further the semen samples with poor fertility records were characterised by a low fructose content (Mann 1954). Besides these, the rate of fructolysis appears to be one of the best methods for assessing not only sperm activity but density as well (Rothschild 1949).

MATERIAL AND METHODS

Studies were conducted on semen made available from the investigations reported in experiment I. In this experiment initial fructose content (IFC), the rate of fructolysis and methylene blue reduction time (MBRT) of semen were determined according to the methods described by Roy *et al.* (1950).

RESULTS

The averages of these seminal attributes, e.g. the initial fructose content (IFC) rate of fructolysis and MBRT, together with the results of statistical analysis during

¹ Experimenters : A. Roy, D. P. Mukherjee, S. N. Luktuke and P. Bhattacharya.

the entire experimental period are presented in Table II. The IFC, rate of fructolysis and MBRT, along with sperm concentration are shown in Table III.

TABLE II

Summary of statistical analysis of average IFC, rate of fructolysis and MBRT of semen of goats

	Control group	Experimental group	Difference between the two means	t-value
IFC (mg./100 ml. of semen)	945.35 \pm 50.40	754.83 \pm 61.29	190.52	2.42**
Fructolysis (mg./100 ml. of semen)	41.34 \pm 2.99	51.70 \pm 3.92	10.36	2.13*
MBRT (minutes)	34.77 \pm 1.75	31.73 \pm 1.90	3.04	1.16

**Significant at two per cent level.

*Significant at 5 per cent level.

TABLE III

Different ranges of sperm concentration and their IFC, fructolysis and MBRT

Range of sperm concentration (in millions/ml.)	Number of observations	Mean of sperm concentration	Mean of IFC (Mg./100 ml. of semen)	Mean of fructolysis (Mg./100 ml. of semen)	Mean of MBRT (Minutes)
0—2999	45	2544.29 \pm 61.39	1294.66 \pm 43.87	23.57 \pm 1.84	45.53 \pm 2.56
3000—3999	86	3497.23 \pm 33.73	1179.15 \pm 40.72	32.59 \pm 1.95	41.55 \pm 2.18
4000—4999	72	4490.72 \pm 21.51	1064.27 \pm 50.28	34.13 \pm 2.27	33.42 \pm 1.95
5000—5999	39	5407.07 \pm 86.65	760.95 \pm 63.59	53.28 \pm 4.52	28.79 \pm 3.19
6000—7999	34	6789.82 \pm 87.70	371.29 \pm 46.60	86.76 \pm 4.01	13.24 \pm 1.59
8000 and above	13	9725.61 \pm 400.28	175.61 \pm 30.40	100.00 \pm 0.00	9.37 \pm 1.50

It may be seen from the tables that when the averages for the whole experimental period were taken into account, the initial fructose content and the rate of fructolysis of the two groups differed as in the case of sperm concentration. In the treated group there was a significant decrease in the initial fructose content while there was a significant increase in the rate of fructolysis. The difference in the methylene blue reduction time of the two groups was not significant even when the average for the whole experimental period was considered.

It will be evident from the tables that with the increase in the sperm concentration per unit volume (i) the initial fructose content per 100 c.c. decreased, and (ii) the rate of fructolysis showed a concomitant rise and (iii) the time required to reduce methylene blue became less. Correlation coefficients between sperm concentration and the above three semen characteristics are given below :

Correlation between sperm concentration per unit volume of semen and

- (i) IFC per 100 c.c. semen -0.968
- (ii) MBRT -0.958
- (iii) Rate of fructolysis $+0.958$

These correlations are highly significant.

From Table IV it can be seen (that with the increase in initial motility there was (i) an increase in sperm concentration and in the rate of fructolysis and (ii) a decrease in the initial fructose content and in the MBRT of semen and that the largest number of semen samples showed an initial motility of $+++$. The difference in sperm concentration and MBRT between $+++$ and $++++$ samples while significant at 2 per cent level but the difference in the IFC and rate of fructolysis were significant only at five per cent level. Differences between samples with $+++$ and $++++$ initial motility were highly significant in all the four characteristics.

DISCUSSION

In an attempt to put the relation of sperm concentration to the degree of fructolysis on a quantitative basis it was found that the ranges of sperm concentration and fructolysis were significantly correlated. The correlation between IFC and sperm concentration was also found to be significant. This higher correlation coefficient, therefore, indicated that for the estimation of sperm concentration a high accuracy existed in the measurement of IFC and fructolysis. This was found to be so, not only in goats but also in rams and buffalo (Roy *et al.* 1950).

The present finding that semen samples with higher sperm concentration and initial motility reduce methylene blue much faster than those with lower sperm concentration and initial motility, confirms that of Van Demark *et al.* (1945) in bull semen.

Warwick *et al.* (1948) found that semen obtained from rams receiving 0.5 g. of thyroprotein, had higher initial motility and reduced methylene blue faster than samples obtained from either the control group or the group receiving 1.5 of thyroprotein. The results obtained in the present experiment show that the average MBRT of the experimental group did not differ significantly from that of the control group. This is due possibly to the fact that MBRT like initial motility of spermatozoa is not a precise method of appraising semen quality and can only be used for differentiating semen samples of widely varying qualities. When the variation is small, the evaluation of quality even within reasonable limits by the use of this method in our experience is a difficult task indeed.

EXPERIMENT ON BUFFALO BULLS ¹

Material and Methods

Twelve buffalo bulls varying in weights from 600 to 800 lbs. and kept under uniform dietary and management regime during the entire experimental period were used for the experiment. Before the experiment started the animals were

¹ Experimenter : S. B. Goswami from thesis for Ph.D.

TABLE IV

Variations in sperm concentration, IFC, fructolysis rate and MBRT of semen in relation to different grades of initial motility of spermatozoa

	Different grades of initial motility				Difference between the means of + + + + and + + + + +	t-value	Difference between the means of + + + + + and + + + + +	t-value
	+ + + +	+ + + +	+ + + +	+ + + + +				
Sperm concentration (in million/ml. of semen)	3119.83 ± 173.94 (24)	3903.84 ± 83.04 (166)	6194.37 = 146.59 (89)		784.0	3.43**	2390.58	14.6**
IFC (in nrg./100 ml. of semen)	1367.62 ± 65.12 (24)	1153.78 ± 29.43 (166)	544.11 ± 38.53 (89)		213.84	2.62*	609.67	12.4**
Fructolysis (in mg./100 ml.)	24.00 ± 3.04 (24)	30.57 ± 1.15 (166)	69.50 = 3.36 (89)		6.57	2.014*	38.93	13.3**
MBRT (in minutes)	42.29 (24)	39.09 (166)	21.84 (89)		10.20	2.36**	17.25	6.62**

*Significant at five per cent level.

**Significant at two per cent level.

Figures in brackets are numbers of samples

TABLE V

Range of variation and the mean of reaction-time and semen characteristics of buffalo bulls during the pretreatment, treatment and the post-treatment period.

Reaction time	Group	Pre-treatment period		Treatment period		Post-Treatment Period	
		Range	Mean	Range	Mean	Range	Mean
Reaction Time in Seconds							
	C	18.0 - 59.8	38.0 (34)	10.5	34.3	14.8 - 43.5	23.0 (76)
	TH	14.6 - 22.1	19.85 (35)	11.0	28.6	9.3 - 54.0	22.9 (78)
Volume							
	C	1.24 - 2.09	1.81 (35)	1.62	2.54	1.20 - 2.16	1.76 (76)
	TH	1.21 - 2.32	1.68 (35)	1.08	2.12	0.97 - 2.16	1.38 (78)
Initial Motility of Spermatozoa							
	C	1.5 - 2.7	2.07 (35)	2.0	3.2	2.0 - 3.4	2.75 (72)
	TH	2.0 - 2.5	2.20 (35)	2.2	3.4	2.5 - 3.7	3.28 (75)
Sperm concentration in millions/ml.							
	C	536.6 - 796.0	721.0 (35)	418.0 - 870.8	605.0 (81)	231.6 - 1333.0	726.5 (72)
	TH	575.8 - 879.1	713.6 (35)	355.8 - 835.8	587.8 (80)	481.6 - 1394.1	751.0 (75)
Total sperm in millions/ejaculate							
	C	710.9 - 1861.5	1321.2 (35)	607.2 - 1638.5	1154.7 (81)	346.3 - 2623.6	1418.6 (72)
	TH	553.3 - 2153.0	1275.0 (35)	436.8 - 1558.9	1029.2 (80)	858.7 - 1950.7	1522.5 (75)
Initial fructose content in mgsm/ 100 ml. of semen							
	C	472.2 - 592.6	543.4 (25)	664.3 - 950.2	821.9 (82)	793.6 - 1054.4	902.6 (71)
	TH	551.3 - 592.8	578.3 (25)	767.9 - 948.9	839.8 (80)	755.7 - 1102.9	872.5 (76)
Fructolysis Index							
	C	0.99 - 3.52	1.96 (17)	1.12 - 3.30	1.98 (64)	0.55 - 4.56	1.98 (53)
	TH	0.74 - 2.76	1.93 (24)	1.52 - 3.49	2.31 (76)	1.04 - 3.20	2.03 (67)
% of abnormal spermatozoa							
	C	was not estimated		9.89	25.86	15.82 (81)	20.51
	TH			6.42	22.41	11.83 (80)	17.09 (67)
						7.42	8.60 (71)

C = Control group

TH = Thyroxine group

Figures in parenthesis indicate the number of observations.

trained to mount an anoestrous cow and to ejaculate semen in artificial vagina. The experimental period consisting of pre-treatment—16 weeks, treatment—14 weeks and post-treatment—13 weeks, extended from the fourth week of June to first week of February. The seminal characteristics studied were :

- (1) Volume
- (2) Initial motility of spermatozoa
- (3) Total spermatozoa per ejaculate
- (4) Percentage of abnormal spermatozoa
- (5) Initial fructose content and
- (6) Fructolysis index.

Besides these reaction time was also studied.

Two successive ejaculates were collected from each animal once every week. The animals were allotted at random to two groups of six each; one as control (c) and the other thyroxine treated (TH). Each of the animals of the treated group was administered orally 10 mg. of sodium—L—Thyroxine (GlaxoLab.) per day.

Methods used to study different semen characters etc. were the same as those used for the goat semen.

RESULTS AND DISCUSSIONS

Reaction time :

The results of the statistical analysis of data (not included because of limited space) revealed that the administration of thyroxine did not influence reaction time. This is in agreement with the results of thyroprotein feeding in goats. Turner *et al.* (1943) found that a Toggenburg buck, which showed a definite lack of interest in oestrous does, displayed sexual interest after the feeding of one gram of thyroprotein per day. Anderson (1956) reported that during the period June to August (in Kenya) when semen quality is relatively poorer in bulls, thyroid therapy had no effect on libido, which was initially good. Definite improvement in libido of bulls with some indications of improved fertility were observed on a dosage of approximately 0.5 to 1.0 g. of thyroprotein daily per 100 lbs. of body weight (Reineke 1946). At these levels no losses in condition or other deleterious symptoms were observed. It seems that the lack of sexual urge in males born with hypothyroid condition may be remedied by the administration of relatively less amount of thyroprotein. But the hypothyroid condition created in males by external factors, such as high air temperature, high relative humidity and high rainfall, does not reduce the sexual behaviour of the animal. The bovine may tolerate a considerably larger dosage of thyroprotein than was employed in Reineke's series. These animals absorb thyroxine from the digestive tract very inefficiently. Reineke *et al.* (1944) have established that it required from approximately 0.5 to 2.g of thyroprotein per 100 lbs. body weight to produce thyroidal effects in cattle.

Semen volume :

Oral administration of thyroxine decreased significantly the volume of semen in buffalo bulls and the adverse effect continued long after the administration of the drug was stopped. This confirms the result obtained in thyroprotein fed goats. It seems that prolonged administration of thyroid substance during summer in this part of the country has some deleterious effect on the semen volume in both goats and buffaloes.

Initial Motility :

Administration of thyroxine significantly increased the initial motility of spermatozoa only during the post-treatment period. Thyroprotein feeding in

goats also had no significant effect on initial motility. Ahmed (1955) also could not find any marked change in the motility of spermatozoa of rams treated with thyroprotein.

The present work on goats and buffaloes and reports on rams indicate that in all the three species administration of thyro-active material has no significant influence on sperm motility. However, thyroid-therapy in bulls in the summer brought about a highly significant improvement in motility. But other bulls with poor motility and high initial pH improved in both respects (Anderson 1956).

Total number of spermatozoa :

Total number of spermatozoa which was estimated on the basis of sperm concentration per unit volume and volume of semen was not effected by the administration of thyroxine. The result is similar to that in goats. Anderson also did not observe any effect of thyroprotein administration on sperm density in bulls. Reineke (1946) reported that there are also indications that at least in some cases there may be an improvement in spermatogenesis.

Percentage of Abnormal spermatozoa :

There was a significant reduction in the percentage of abnormal spermatozoa in thyroxine-fed animals. This observation agrees with those in rams (Berliner and Warbritton 1937, and Bogart and Mayer, 1946), but contrary to those reported by Eaton *et al.* (1948) who found increase in the percentage of abnormal spermatozoa in rams fed with thyroprotein. There was also no significant effect of thyroprotein on the abnormal sperm of goats.

Initial fructose content :

The administration of thyroxine to buffalo bulls resulted in lower initial fructose content and the finding is in conformity with results in thyroxine fed goats.

Fructolysis Index :

Fructolysis index of buffalo semen in thyroxine treated group was significantly increased and the result is in agreement with those reported here in goats.

In conclusion we may suggest that treatment with thyroid material is not advisable for all sterility or infertility problems in males. Before applying thyroid therapy to such animals it may be necessary to select individuals deficient in thyroid function for obtaining satisfactory response to such treatment.

Moreover thyroid therapy necessitates thorough investigations into the degree of hypothyroid condition in different species and breeds of livestock in different geographical regions of the globe so widely varying in climatic conditions even in the same season.

CONCLUSIONS

Experiments on male goats :

Thyroprotein (Protamone) feeding at a level of 1 gm. per day per animal to male goats over a period of three months (from the middle of May to the middle of August) had no significant effect on the reaction time, initial motility and total number of spermatozoa per ejaculate and percentage of abnormal spermatozoa. A highly significant decrease in semen volume was observed due to the administration of the drug with a concomitant increase in sperm concentration. Thyroprotein feeding had no significant effect on methylene blue reduction time of semen. Initial fructose content in semen decreased and the rate of fructolysis increased significantly under the influence of the drug.

Highly significant negative correlation coefficients were found between sperm concentration, initial fructose and methylene blue reduction time. There was highly significant positive correlation coefficient between sperm concentration and rate of fructolysis of semen.

Experiments on buffalo-bulls :

Oral administration of 100 mg. of sodium-L-thyroxine (Glaxo, Lab.) per day to each of the six experimental buffaloes during a period of 14 weeks (August to early November) did not influence the sex libido (reaction time) of the animals. The initial motility of the spermatozoa was significantly improved during the treatment period. The improved motility was maintained during the post-treatment period. The administration of L-thyroxine had an adverse effect on the semen volume and the effect continued during the post-treatment period of thirteen weeks. The drug did not affect the sperm concentration. The initial fructose content in semen was reduced during the treatment period which continued up to the end of the post-treatment period. The fructolysis index was increased during the treatment period. Percentage of abnormal spermatozoa in the ejaculates was reduced during the treatment period and this reduction continued throughout the post-treatment period.

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THE NUCLEAR APPARATUS OF *FRONTONIA LEUCAS* (EHRBG)

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ABSTRACT

The paper deals with a study of the nuclear apparatus of *Frontonia leucas* (Holotricha; Frontoniidae), in the vegetative condition as well as during binary fission. The macronucleus is found in the centre of the cell, and is ovoid. It is strongly Feulgen positive, and divides amitotically during binary fission. In a certain percentage of cases there is elimination of chromatin from the macronucleus as the two daughter nuclei separate. This eliminated chromatin gets absorbed in the cytoplasm of either daughter cell. Micronuclei vary in number from five to twelve and are found as spherical, strongly Feulgen positive bodies around the macronucleus, most of them lying very close to it. They divide mitotically and are the first to show visible signs of binary fission. Their division is synchronous and is completed before that of the macronucleus, and the latter completes its division before the cytoplasm, both nuclei attaining their vegetative stage just before the daughter cells separate.

INTRODUCTION

Frontonia leucas is a fresh water euciliate belonging to the order Holotricha, Sub-order Hymenostomata, family Frontoniidae. It has a single oval macronucleus and five to twelve micronuclei. The few studies made on this animal are mainly morphological. Popoff (1907, 1908), made some growth studies. Tonniges (1914) and Wetzel (1925) described the trichocyst apparatus. Bullington (1925) studied the method and rate of movement of this species together with other ciliates like *Paramecium*, and *Euplotes*. Hood (1927), worked on the zoochlorellae of the animal. Darby (1929) studied the effect of hydrogen-ion concentration on the sequence of this form and also of other ciliates like *Dileptus* and *Blepharisma*. Holter and Doyle (1938) demonstrated the presence of amylase, peptidase and catalase activity in protozoa, which included *Frontonia*, *Paramecium* and *Amoeba*. The present paper is a study of the nuclear apparatus of *Frontonia leucas* in the vegetative condition and during binary fission.

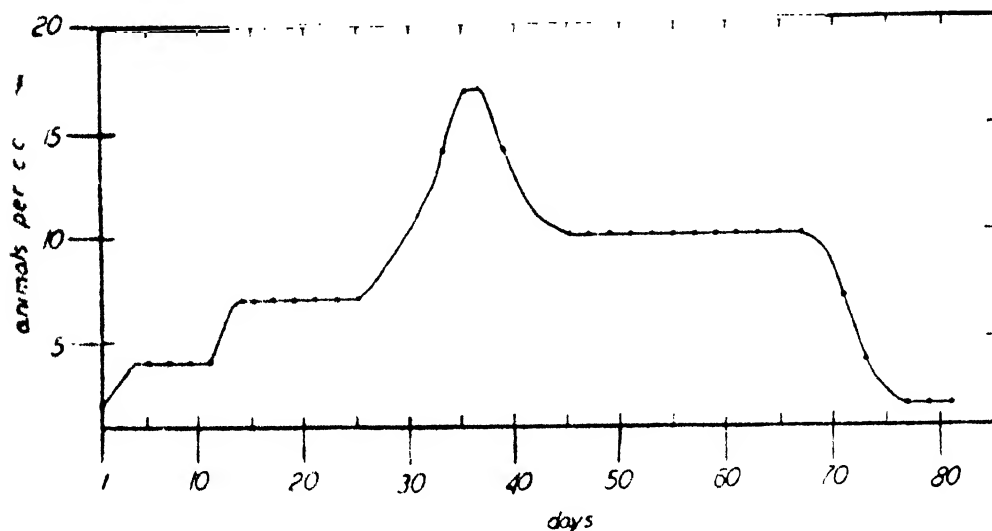
MATERIAL AND METHODS

Kahl's work cites sixteen species of *Frontonia* which include *Sigmostomum indicum* from India.

For the present study, collections were made around Bangalore, from stagnant pools. The animals were identified as *Frontonia leucas*, after the description by Kahl (1930).

A number of media was tried for culturing the animals in the laboratory; the one most favourable was found to be hay infusion to which pieces of sterilised cabbage leaves were added from time to time. *Frontonia* in mixed cultures is found to feed on other ciliates like *Euplotes* and *Paramecium*. They ingest even rotifers, and exhibit cannibalism. We have however been able to maintain cultures of *Frontonia* free of other protozoans. They ingest decayed cabbage leaf particles also and living specimens as well as fixed ones show green materials in the cytoplasm.

pH also has its effect on the growth of the culture and a culture at pH between six and seven thrives well. Temperature and other climatic conditions appear to affect the growth of the culture. During the rainy season, the rate of division is higher. Also, divisions are more common during the cooler parts of the day and night. During periods of active growth, dividing animals are noticed throughout the day, but here again the fission rate is higher towards the evening. On the whole, a cool and pleasant climate seems to favour their growth and division.



Graph showing growth curve of *Frontonia leucas*.

Animals in the same dish were noticed to divide synchronously. Division occurs once in three days during the maximum growth phase of a culture; at other times it takes from five to six days for a fission.

Preparations were made by fixing the animals in Carnoy's fluid and staining with the Feulgen reagent. Light green was used as counterstain.

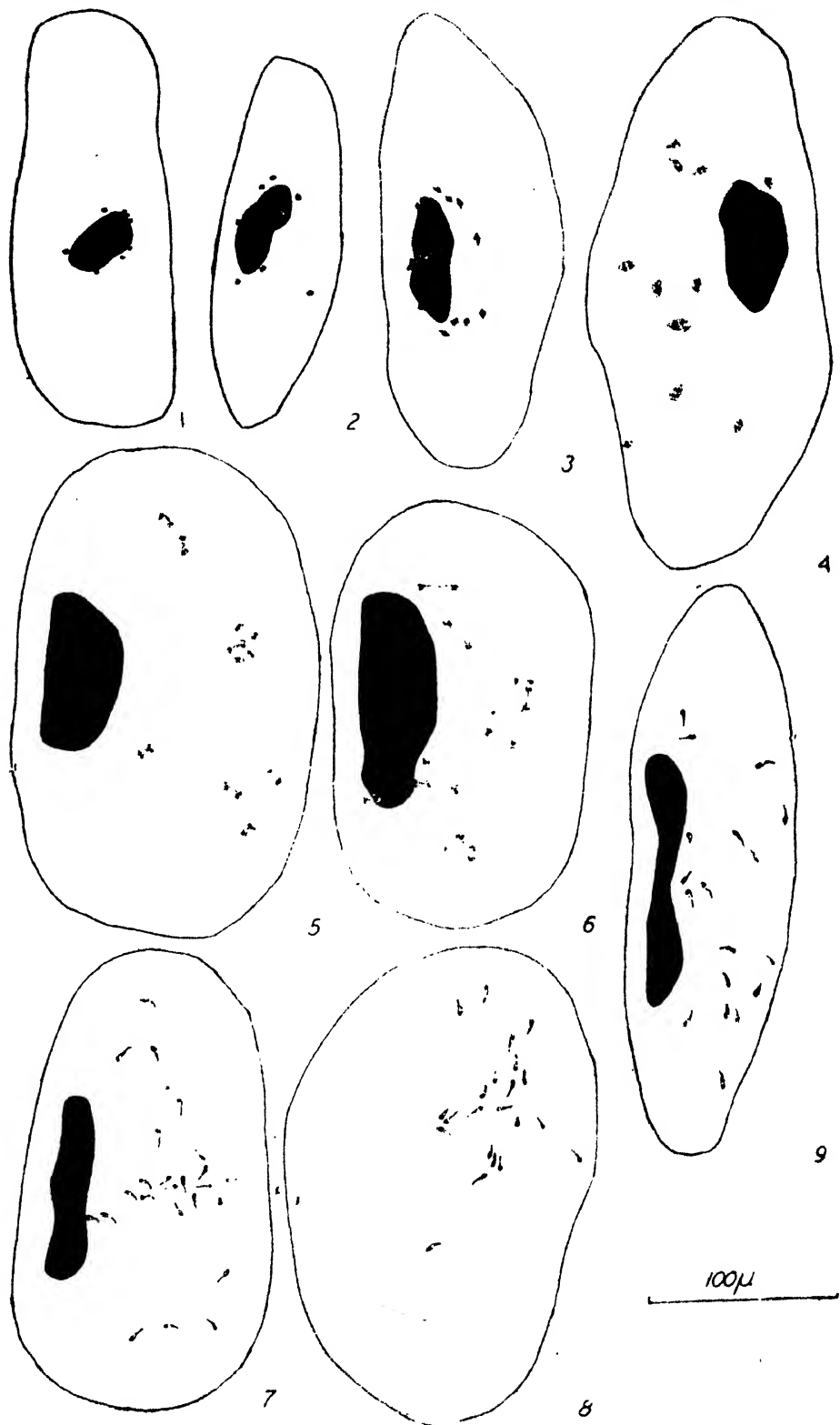
OBSERVATIONS

Frontonia leucas is oval in outline and somewhat flattened. The length of a well fed animal varies from 300μ to 550μ and breadth varies from 150μ to 250μ .

EXPLANATION OF TEXT-FIG. 1.

All figures are camera lucida drawings of Feulgen preparations of *Frontonia leucas*.

- FIG. 1.—Vegetative animal showing the macronucleus and micronuclei.
- FIG. 2.—Shows animal with the micronuclei in prophase.
- FIG. 3.—Animal with slightly elongated macronucleus and micronuclei in metaphase.
- FIG. 4.—Shows macronucleus almost in the same stage as in previous figure with micronuclei in early anaphase.
- FIG. 5.—Animal with micronuclei in late anaphase.
- FIG. 6.—Macronucleus still more elongated with micronuclei in telophase.
- FIG. 7.—Macronucleus in the same stage as in figure 6 with micronuclei which have already finished division.
- FIG. 8.—Macronucleus in the highly elongated stage with micronuclei after division.
- FIG. 9.—Dumb-bell shaped macronucleus with micronuclei in the same stage as in figures 7 and 8.



TEXT-FIG. 1.

The macronucleus is oval in outline and is found towards the centre of the cell. It has a length of 45μ to 65μ and a breadth of 35μ to 50μ . It is strongly Feulgen positive. Micronuclei are small spherical bodies, 2.5μ to 3μ in diameter and strongly Feulgen positive. They are found around the macronucleus, many of them closely adhered to it (Fig. 1).

Growth of animals follows a definite pattern. The method adopted to study the growth is as follows. To reduce experimental error, three different jars containing *Frontonia* cultures were taken and the study was made on an average basis. To start with, there were about two animals per c.c. in 350 c.c. of culture fluid contained in the jar. Animals per c.c. were counted every alternate day, as division, under most favourable conditions, occurs once in three days. This was followed for eleven weeks when the population decreased to a constant phase without any further change, unless the culture medium was changed. Also, if the culture medium is changed from time to time, growth of the animal does not show this type of change from one phase to another. The growth curve shows three constant phases, the first two followed by active growth phases. The third and the longest constant phase is followed by an accelerated death phase. After this the population decreases at a fairly constant rate. This curve is taken in a culture at pH between 6 and 6.5. Atmospheric temperature varied from 25°C to 30°C during the experiment.

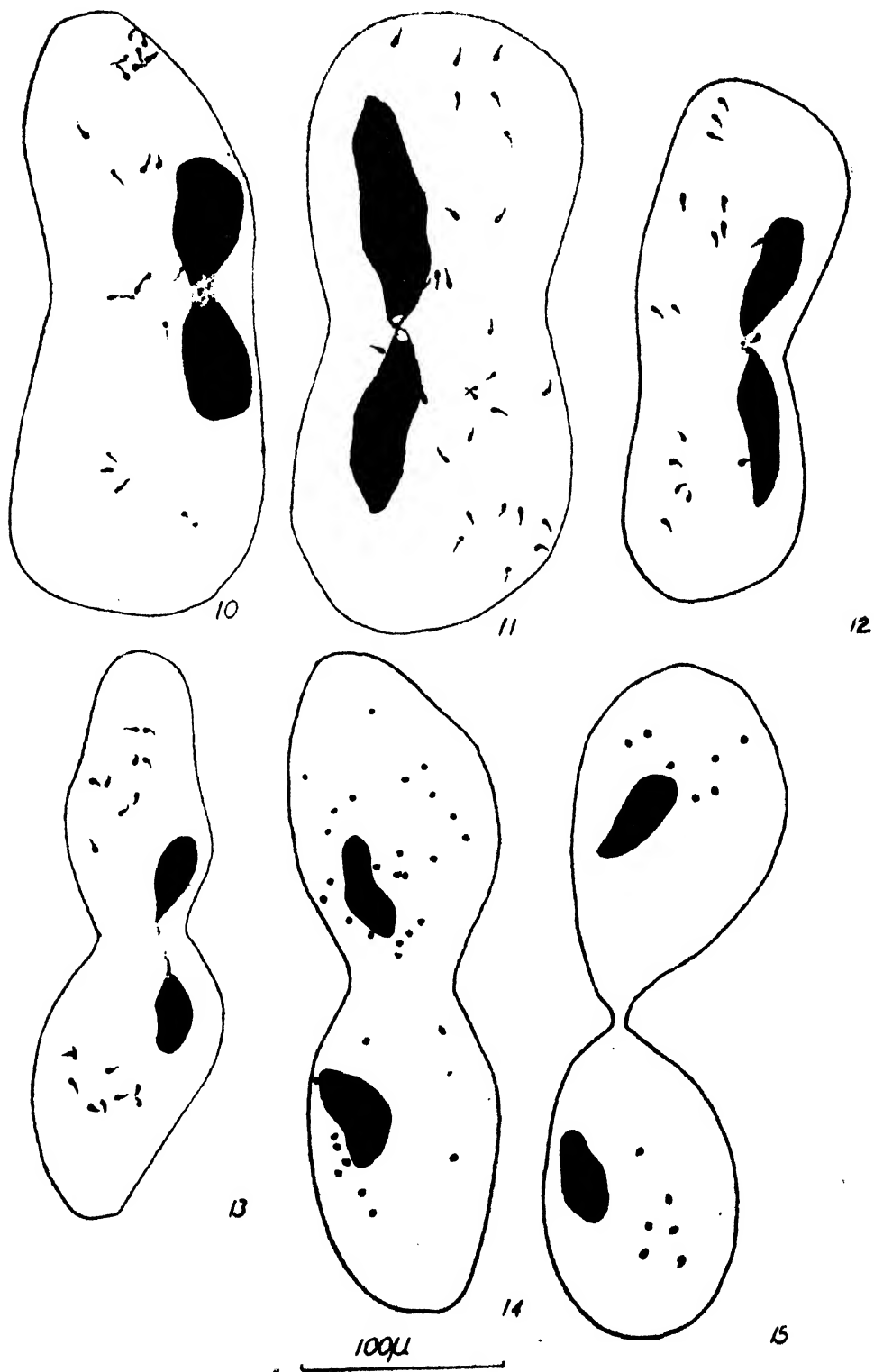
Binary fission. Binary fission is the usual type of reproduction in this species. Under favourable conditions, the animals divide once in three days, but starvation and slight changes in the culture medium reduce the rate of division.

It takes from 30 to 45 minutes for a normal individual to separate into daughter cells, after the appearance of the external cytoplasmic constriction. The dividing animals are less active than vegetative ones, and towards the end of fission, exhibit a rotatory movement, one of the daughter cells twisting about the other.

At the onset of division, the micronuclei increase slightly in size and have a puffed-up appearance. This is prophase. Many of them move away from the macronucleus. The evenness of staining is lost and the micronuclei stain more lightly (Figs. 2 and 20b). At metaphase, the micronuclei become slightly elongated, attaining a spindle shape (Figs. 3 and 20c). The dark staining chromatin material is seen at the equator of the spindle. It has however not been possible to make a count of the chromosomes. In a number of animals, a few micronuclei do not take part in division but degenerate. The spindle elongates further and the chromatin material separates towards the two poles (Figs. 4 and 20d). The spindle element remains as a bundle of thin strands connecting the two groups of chromosomes (Figs. 6 and 20f). Next, the daughter nuclei separate, the spindle having snapped in the middle. The daughter micronuclei appear as elongated

EXPLANATION OF TEXT-FIG. 2.

- FIG. 10.—Dumb-bell shaped macronucleus with a Feulgen negative area in the division region with Feulgen positive granules in it.
 FIG. 11.—Shows a twist in the middle of the Feulgen negative area.
 FIG. 12.—The daughter micronuclei have started segregation and condensation.
 FIG. 13.—The daughter macronuclei move further apart. Micronuclei get segregated on either side of the dividing cell. There is equal distribution of micronuclei in this particular animal. Cytoplasm shows constriction on both sides.
 FIG. 14.—Macronuclei are slowly condensing and assuming their vegetative shape. Animal shows unequal distribution of micronuclei.
 FIG. 15.—Macronucleus has moved to the centre of either daughter cell. Cytoplasmic constriction has deepened.



TEXT-FIG. 2.

bodies distributed in the cytoplasm, remains of the spindle material trailing off at their more pointed ends. The broader part of the separated micronuclei stains more deeply than the pointed (Figs. 7, 8, 9, 10, 11, 12, 13 and 20g, h). This phase in division lasts for a considerable time, until the macronucleus, which, at this time shows the first constriction, divides and the two parts move apart.

As the macronuclear halves move apart, the micronuclei get segregated on either side of the dividing cell. They condense, move towards the macronucleus and get arranged close around it as in the vegetative animal (Fig. 16). The evenness of staining is regained.

A phenomenon of interest noticed in connection with the division of the micronuclei is the unequal distribution of the products of micronuclear division among the daughter cells. The difference is often so high that one of the daughter cells has a number of micronuclei twice that of the other (Fig. 15).

The macronucleus divides by amitosis. The first visible changes are noticed when the micronuclei are in prophase or early metaphase. It gets elongated, reaching a length varying from 120μ to 130μ in the different animals studied (Figs. 8 and 9). A constriction appears in the middle and the nucleus assumes a dumb-bell shape (Fig. 10). The constricted part stains more lightly than the rest of the nucleus and at a later stage, is entirely Feulgen negative, except for a few Feulgen positive granules. The nuclear membrane is intact and connects the two daughter nuclei about to separate (Fig. 10). A twist appears in the middle of this constriction (Fig. 11) and soon the daughter nuclei separate (Fig. 12). The division of the macronucleus is completed before that of the cytoplasm and by the time the external constriction of the cytoplasm appears, the two daughter macronuclei have separated (Fig. 13). As they move towards either side of the dividing cell, they attain the vegetative shape and size (Fig. 16).

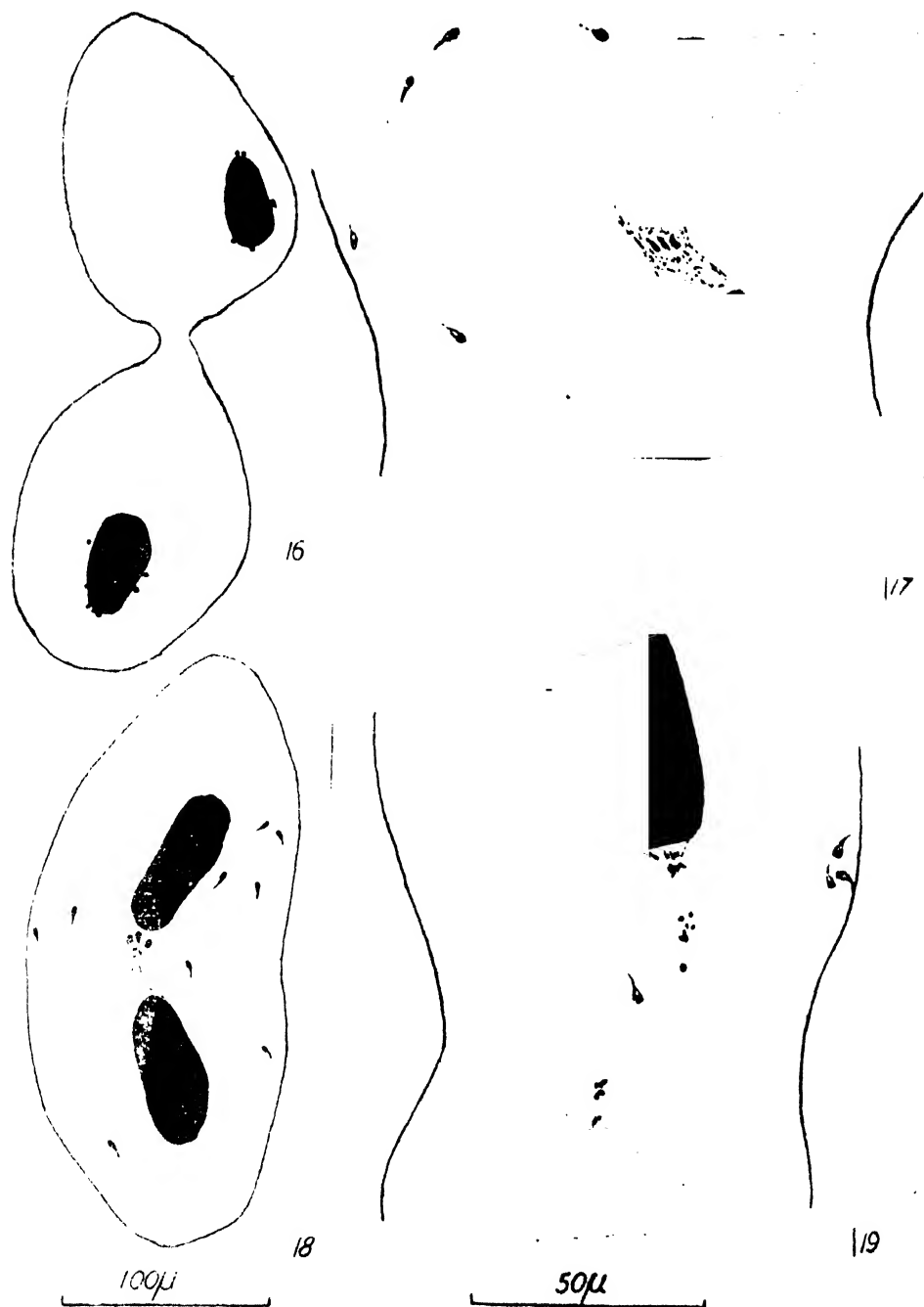
Perhaps the most interesting feature regarding macronuclear division is the elimination of chromatin seen in about 40 per cent of the cases studied. The amount of material eliminated varies in different individuals, elimination taking place at the time the two daughter nuclei separate (Fig. 18). In all cases the eliminated chromatin gets fragmented (Fig. 19) and lost in the cytoplasm of either daughter cell. Elimination has been noticed in a higher frequency when division rate is high.

The division of the macronucleus precedes that of the cytoplasm, which takes from 30 to 45 minutes after the external appearance of the cytoplasmic constriction. The constriction first appears on one side of the animal and later extends to the other side also. When the cytoplasmic constriction is clearly seen on either side of the dividing animal it is an indication that the macronucleus has completed its division.

DISCUSSION

Two special problems arise in connection with fission in *Frontonia leucas*: (1) Unequal distribution of micronuclei among the daughter cells, (2) Elimination of chromatin during macronuclear division.

Unequal distribution of micronuclei results in the production of vegetative animals differing in their micronuclear number, often strikingly. Such unequal distribution is seen in 50 to 55 per cent of the animals studied. The significance or consequence of this is not known. Moreover, when it results in an individual with so high a number as 20 as compared with the other cell which receives only 10 or 12, the explanation becomes all the more difficult. One suggestion to explain this phenomenon is that when the number of micronuclei is very large, the mechanism of segregation gets out of control either because it is not very well organised or because it is put under a greater strain than is expected



TEXT-FIG. 3.

- FIG. 16.—Macronucleus in each cell has attained the vegetative form with the micronuclei arranged around it.
 FIG. 17.—The constricted Feulgen negative division region of the macronucleus, showing Feulgen positive granules in it. Nuclear membrane intact.
 FIG. 18.—Animal showing elimination of chromatin, as the daughter macronuclei separate.
 FIG. 19.—Shows fragmented granules of the eliminated chromatin.

of it. It is noticed that wherever the initial number of micronuclei is very high, the daughter cells differ to a much greater extent in their micronuclear number. Another point which requires an explanation in this connection is the extreme rarity of vegetative animals with so a high number of micronuclei as 23. It is possible that some micronuclei get lost or absorbed in the cytoplasm during reorganisation after division.



TEXT-FIG. 4.

FIG. 20.—(a) Vegetative micronuclei, (b) Micronuclei in prophase, (c) Micronuclei in metaphase, (d) Micronuclei in early anaphase, (e) Micronuclei in late anaphase, (f) Micronuclei in telophase, (g) Micronuclei after division.

The elimination of chromatin from the macronucleus is reported in a number of protozoa. Calkins (1919) has observed in *Uroleptus halseyi*, each of the eight macronuclei is "purified" by discarding a reorganisation band and an "X-body" into the cytoplasm before fusing into a single body which then divides into two nuclei. According to Kudo (1950), in *Loxocephalus*, *Eupterion* and *Endamoeba blattae*, during the nuclear division, there appears and persists a small body within the nuclear figure located at the division plane. Kidder (1933) observed that *Conchophthirius mytili* casts off a part of its chromatin during every vegetative division, which later gets absorbed in the cytoplasm of either daughter organism. Kidder has also observed the phenomenon in other species of *Conchophthirius*. Kidder and Diller (1934) reported chromatin elimination in *Glaucoma scintillans*, Kidder and Summers (1935) in *Allosphaerium*, and Suzuki (1954), in *Blepharisma undulans americanus*. It is believed to be a process where waste substances accumulated in the nucleus during vegetative life are cast off.*

No reorganisation band has been recognised in ciliates with a more or less rounded macronucleus (Kudo, 1950). In *Frontonia leucas* during division the macronucleus

shows a weak staining region in the division plane, with a few Feulgen positive bodies in it (Fig. 17). In a few cases, these bodies were found in three groups (Fig. 18), recalling remarkably the reorganisation band. This is particularly noticeable before chromatin elimination. The chromatin elimination is of importance in the life of this ciliate as this might account for the extremely infrequent occurrence or even complete absence of conjugation and related phenomena by which other ciliates reorganise their macronucleus. During the past two years the cultures have been maintained in our laboratory, conjugation has never been observed in our material. The suggestion that chromatin elimination could be a method of reorganising the macronucleus appears plausible, as the phenomenon is more frequently met with in cultures where the division rate is high. When successive divisions are closer to each other than usual, the time interval between one division and the next is too little for the animal to reorganise its macronucleus. Hence the elimination after reorganisation takes place at the end of three or four divisions instead of after every division.

Kidder and Diller (1934) also report that elimination of chromatin in *Colpidium* and *Glaucoma* does not occur in a few divisions subsequent to reorganisation after conjugation. They attribute this to the high division rate and infrequent occurrence of conjugation in *Colpidium* and *Glaucoma*. In the light of the above, the suggestion that this phenomenon of chromatin elimination during the division of the macronucleus in *Frontonia leucas* is an attempt to reorganise the macronucleus, seems plausible.

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CHARACTERISTICS OF THE SAPROPHYTIC CORYNEFORM BACTERIA

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ABSTRACT

A collection of a hundred and fourteen strains of saprophytic coryneform bacteria (genus *Arthrobacter*) representing seventy-one fresh isolates and forty-three strains received from other investigators has been subjected to a comparative systematic study. The characteristics that reliably distinguish the group as a whole are its primary habitat, inability to produce catalase and indole and general weakness for carbohydrate metabolism. In the further differentiation of the genus into species, the limitations of relying exclusively on physiological characteristics have been exposed. The usefulness of Robinow's cell-wall staining technique in morphological and systematic studies on the group is emphasised. It is tentatively proposed that a taxonomic scheme which would primarily divide the genus into morphological groups and use physiology for further differentiation, may be more satisfactory than the present scheme.

Though the existence of saprophytic bacteria morphologically related to the parasitic *Corynebacterium* has long been recognised (Kisskalt and Berend, 1918), it is only in recent years and largely due to the work of Lochhead and co-workers that the growing significance of these organisms has been realised (Lochhead, 1952; Lochhead and Burton, 1956). The abundant occurrence of these organisms in soil is now well authenticated and they have also been isolated by enrichment culture techniques that imply their role in the utilization of asparagine, hippurate, glycerol, starch and cellulose (Indian Council of Agricultural Research, 1957). It has long been suspected that the *Corynebacterium*—*Nocardia* group of bacteria normally carry out the oxidative degradation of pyrimidines in a mineral environment. They have been implicated in the breakdown of hydrocarbons (Trecanni, 1954; Ladd, 1955), acetone (Levine and Kramptiz, 1952) and organic nitro compounds (Gundersen and Jensen, 1956). Nevertheless a special ecological niche has yet to be assigned to them.

Jensen's admirable review (1952) has served to emphasize the obscurity which has shrouded the systematics of this group of bacteria, a position which has not been entirely mitigated despite the accordance of generic status (genus *Arthrobacter*) to the group in the new edition of Bergey's manual (Breed *et al.*, 1957). The determinative taxonomy of any group ultimately depends on careful studies and descriptions on as large a number of strains within the group as possible. In the present state of our knowledge, the need for the accumulation of more information on strains of coryneform bacteria needs no emphasis. The comparative studies reported in this paper on a collection of 114 strains comprising 71 fresh isolates and 43 strains received from other investigators, is intended to partly fulfil this need.

METHODS

Source of isolates. The majority of strains were freshly isolated from samples of garden soil and the rhizosphere of a variety of plants. Primary isolation was carried out on one of the following media: soil extract agar (Lochhead and Chase, 1943), nutrient agar and nutrient agar containing 1.0 per cent soil extract and 0.1 per cent yeast extract. In addition to these, isolates were also received for study from other investigators interested in this group of bacteria.

Morphological Studies. The developmental morphology of each strain was studied using *in situ* preparations of the organisms growing between the glass surface of a slide and a thin layer of overlaid sterile nutrient agar cut out from a previously poured petri-dish, the slides being incubated in a moist chamber at 27–29°. At desired intervals, slides were fixed in Bouin's fixative, the agar layer carefully peeled off and the adherent cells stained by the tannic acid-crystal violet technique of Robinow (1942) to demonstrate cell wall and septa. Observations were also made on monochrome and Gram stained smears prepared in the conventional manner from growth on the following media: nutrient agar, nutrient agar with 1.0 per cent sodium chloride, nutrient agar with 1 per cent glucose, soil extract agar and Ashby's nitrogen-free mannitol agar. The use of different media as a possible aid in the morphological differentiation of coryneform bacteria has been suggested by Gibson (1953). The developmental morphology of all strains was studied over a period of seven days. The motility of all strains was tested in young (18 hours) as well as in old cultures (4 days) grown on nutrient agar. For the conclusive demonstration of motility in doubtfully motile organisms the method of Tittler and Sandholzer (1936) was employed. Löffler's method (Buchanan and Buchanan, 1938) was found satisfactory for staining of flagella.

Colony characteristics of all the strains were studied on nutrient agar. Growth characteristics in nutrient broth were also observed. The optimum and limiting temperatures and hydrogen ion concentration for growth were determined in nutrient broth.

Physiological Tests. All conventional physiological tests that have been found useful in classification of the better known groups of bacteria were used. Unless otherwise stated, these tests were carried out according to the recommendations of the Society of American Bacteriologists (1957) in their *Manual of Microbiological Methods*. Inasmuch as these tests were found to be of limited value, several additional physiological tests were also used.

Carbohydrate metabolism. The oxidative or fermentative nature of the organism in relation to the carbohydrates arabinose, xylose, glucose, fructose, galactose, sucrose, maltose, lactose, inulin, glycerol, mannitol and sorbitol was tested according to the method of Hugh and Leifson (1953). This test replaced routine fermentation tests.

Reduction of methylene blue. This test was carried out according to the recommendations of Smith *et al.* (1952). The composition of the medium was as follows: glucose, 5.0 g.; proteose peptone, 10 g.; agar, 2.5 g.; methylene blue, 0.004 g.; distilled water, 1 litre. Tubes 15 mm. in diameter containing 8.0 ml of the semisolid medium were inoculated and examined for the reduction of methylene blue after 1, 3, 5, 7, 14 and 21 days.

Anaerobic production of gas from nitrate. A modification of Gibson's medium (Gibson, 1944) suggested by Smith *et al.* (1952) was used. The medium contained tryptone, 1.0 g.; K_2HPO_4 , 0.5 g.; meat extract, 0.3 g.; yeast extract, 0.2 g.; $NaNO_3$, 1.0 g.; agar 0.5 g.; distilled water, 100 ml. The pH was adjusted to 7.6. Approximately 8.0 ml of the medium in 15 mm. tubes was autoclaved, quickly cooled to remove oxygen, inoculated and capped with 10–15 mm. of sterile vaspar. The tubes were incubated at 27–29° and observations made up to 15 days.

Production of urease. This was demonstrated as follows: growth from nutrient agar slants was washed off with about 4.0 ml. of sterile distilled water. Using phenolphthalein as indicator, the pH of the suspension was adjusted to 7.0. The suspension was divided into two equal parts, one being the control and to the other, about 0.1 g. of urea (crystals) was added. The development of an alkaline reaction indicated the production of urease. Tests were done after 5, 10 and 15 days.

Utilization of carbon and nitrogen compounds. Knight and Proom (1950) after an extensive investigation on the genus *Bacillus*, suggested that nutritional surveys on bacterial groups would give indications of natural relationships tha

would make classification less arbitrary than it is at present. The isolates in our collection were examined for their ability to utilize a number of carbon and nitrogen compounds. Throughout the course of these tests, special precautions were taken to avoid traces of unwanted nutrients. The selection of test compounds was governed by available information on the physiological and metabolic activities of these microorganisms. The composition of the basal medium used in these tests was as follows: neutral phosphate mixture (Na_2HPO_4 , 8.0 g.; KH_2PO_4 , 2.0 g.), 1.0 g.; NaCl , 0.05 g.; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g.; $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$, (saturated solution) 5.0 ml.; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 2.5 mg.; micronutrient solution 1 ml.; distilled water, 1 litre. The composition of the micronutrient solution used was as follows: $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 11.0 g.; $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 5.0 g.; CoSO_4 , 0.05 g.; H_3BO_3 , 0.05 g.; Na_2MoO_4 , 2.0 g.; $\text{CuSO}_4 \cdot 7\text{H}_2\text{O}$, 0.007 g.; distilled water, 1 litre. To this medium 0.5 g. ammonium sulphate was added as the nitrogen source and the following carbon compounds added individually in the concentrations specified: phenol, 0.1% (v/v); asparagine, benzene, aniline, o-cresol, m-cresol, alpha-naphthol, beta-naphthol, paraffin, acetone, benzaldehyde, anthracene, and caffeine, 0.1% (w/v); naphthalene, 0.0003% (w/v) and the sodium salts of the following acids: formic, oxalic, oleic, propionic, succinic, benzoic, and salicylic, 0.05% (w/v); barbituric 0.01% (w/v); hippuric, 0.1% (w/v). acetic, citric and lactic, 0.5% (w/v).

The following inorganic nitrogen compounds tested were added to give a concentration of 0.5% (w/v), using 0.5% glucose as the carbon source: NaNO_3 , $(\text{NH}_4)_2\text{SO}_4$, $(\text{NH}_4)_2\text{HPO}_4$, and NH_4NO_3 .

The utilization of amino acids was tested when provided as the sole source of carbon and nitrogen and also when provided as the nitrogen source only. The various amino acids were incorporated so that the final volume contained 25 mg. of nitrogen per ml. The following amino acids were sterilized by Seitz filtration and added separately to the medium: dl-glycine, dl-alanine, dl-serine, dl-norleucine, dl-threonine, l-leucine, dl-valine, dl-isoleucine, dl-aspartic acid, dl-glutamic acid, L-arginine, dl-lysine, L-asparagine, l-histidine, L-proline, L-hydroxyproline, dl-tryptophane, L-tyrosine, dl-methionine, and L-cystine.

The total volume of defined medium in every case was 5.0 ml. The inoculum consisted of washed cells harvested from a 24 hour old nutrient agar slant. The inoculated tubes were incubated at 27-29° and growth recorded visually up to a period of seven days.

Thermal death time. This was determined in nutrient broth containing 10^3 to 10^4 viable cells per ml., the time being recorded after the contents of a 'parallel' tube reached the desired temperature.

RESULTS

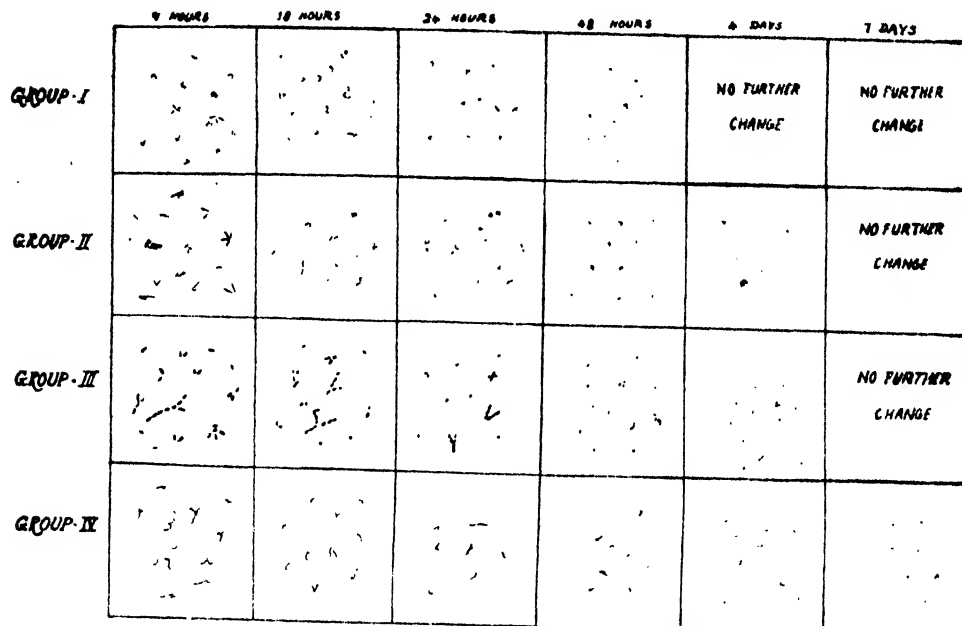
Morphology and growth characteristics

The gross morphological characteristics were generally similar in most of the media utilized. Nevertheless, the following observations were made:

- 1—Growth was good to luxuriant on both nutrient agar and glucose nutrient agar, but poor on soil extract agar and Ashby's mannitol agar.
- 2—Pigmentation was more pronounced on glucose nutrient agar than on nutrient agar, soil extract agar or Ashby's mannitol agar.
- 3—The organisms showed vacuolation earlier on soil extract agar and Ashby's mannitol agar than on nutrient agar or glucose nutrient agar.
- 4—The tendency to branching was maximum on glucose nutrient agar followed by nutrient agar, Ashby's mannitol agar and soil extract agar in that order.

With the help of morphological characteristics as revealed by monochrome and cell wall staining, it was possible to divide the isolates in the collection into four morphological groups. The morphological distinctions between the four groups are presented diagrammatically in Text-figs. 1 and 2.

Group—I. Organisms in this group were of the smallest size and coccobacillary in shape. 'V' forms were encountered but a more pronounced tendency was to



TEXT-FIG. 1.

Developmental morphology of the four morphological groups of saprophytic coryneform bacteria as observed in the crystal violet-stained smears from nutrient agar ($\times 1200$).

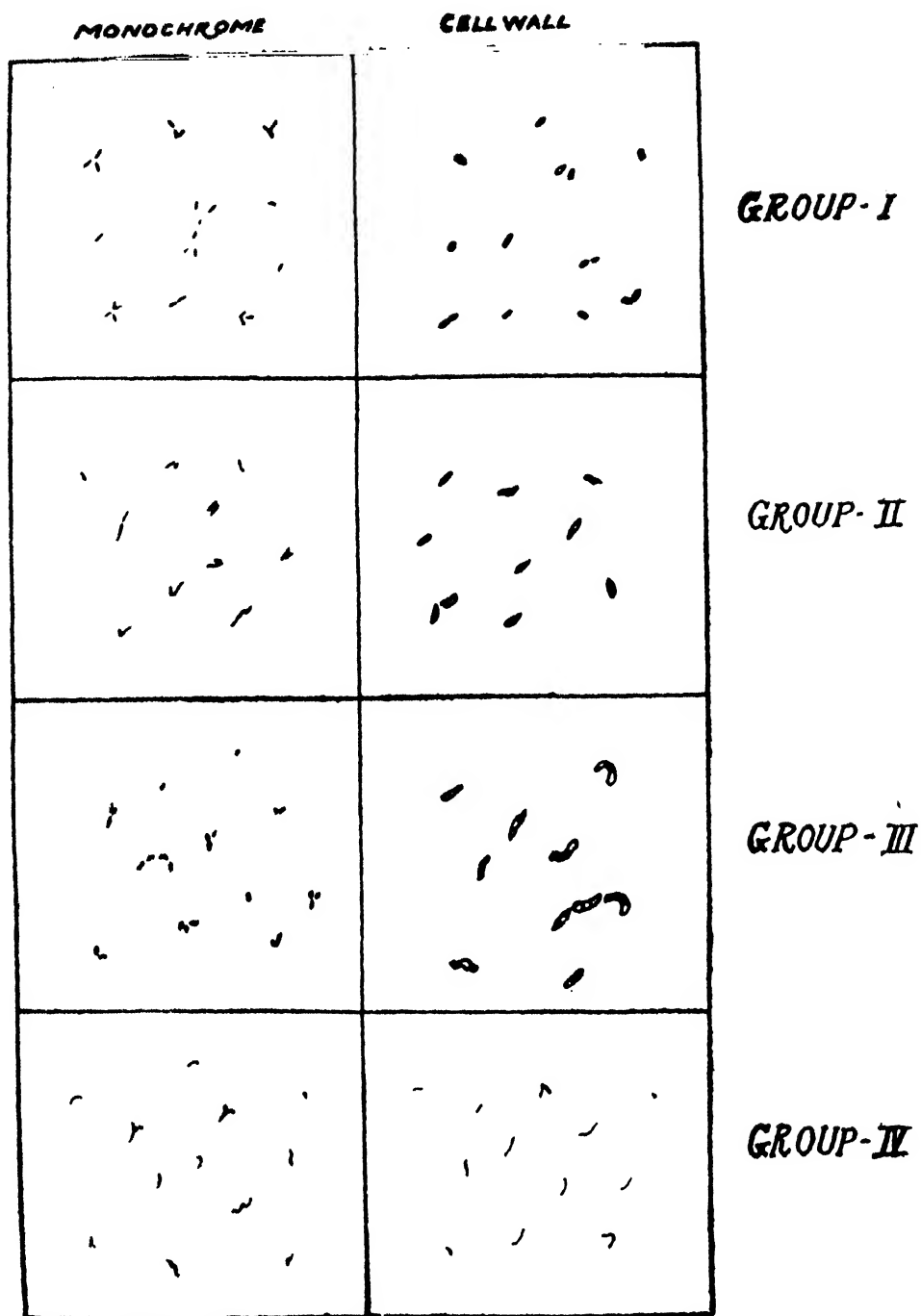
form clusters. On cell wall staining, these bacteria did not show the presence of any septum but only deep staining at the ends, thereby appearing as bipolar rods. The organisms became almost coccoidal at the end of 24–36 hours growth on nutrient agar. Gram staining showed that most of the cultures in this group were Gram positive to Gram variable. All the strains were distinctly Gram positive at the end of the fourth day when they were in the coccoidal stage. Excepting three strains which were motile by means of a single polar flagellum all other strains in this group were nonmotile (see Table 1).

TABLE I
Motility and type of flagellation

Morphological group and number of strains in each group	Number of motile strains	Type of flagellation		
		Single polar	More than one polar flagellum	Peritrichous
I (13)	4	4	—	—
II (49)	24	14	4	6*
III (28)	15	10	—	5**
IV (24)	14	9	5	—

*Includes 2 amphitrichous and 1 lophotrichous strains

**Includes 3 strains showing only lateral flagellation.



TEXT-FIG. 2.

Distinctions between the four morphological groups as revealed by cell wall and monochrome staining of 18 hour old cultures. ($\times 1500$).

Pigmentation on suitable solid media was generally yellow, but two strains were orange, one pinkish red, and four pearly white. The colonies on nutrient agar were small, round, entire, slightly raised, translucent to opaque, smooth and glistening. The size varied between 0.5 mm.—1.0 mm. In nutrient broth the strains generally showed uniform and moderate to good turbidity and a smooth and powdery sediment. Only three strains showed a compact sediment and no turbidity (Table 2). The pH range for growth was between 6–8.5 though two strains could grow at pH 4.5. The optimum temperature for growth was between 25–30° and the maximum temperature was 40° except for four strains which failed to grow beyond 37°.

Group—II. This group consisted of organisms of variable size ranging from small coccobacillary forms to distinct rod-shaped organisms of the size about $0.5\text{--}0.8\mu \times 1\text{--}3\mu$. The smaller rods had a tendency to form clumps with the occasional presence of 'V' and 'L' forms. The larger rods showed a more pronounced tendency to palisade arrangement and angular forms. They showed a distinct tendency to filament and chain formation in young cultures. The smaller rods broke up into coccoidal forms by the end of 48 hours whereas the larger forms required three to four days. Cell wall staining revealed the presence of a single septum. The method of division and the resulting arrangement was not clearly revealed in the case of smaller forms by the cell wall staining technique even when viewed under phase contrast. In the case of larger forms the cells after division presented mostly an 'opposite angle' arrangement (Sundman, 1958). The organisms were weakly to distinctly Gram positive in young cultures; the coccoidal stage was always distinctly Gram positive. Fortynine strains belonged to this group of which twenty-six were nonmotile and the remaining twenty-three motile. Among the motile strains flagellation was predominantly single polar, though a few showed lateral, amphitrichous, lophotrichous and peritrichous flagella (see Table 1).

Pigmentation varied from grayish and pearly white to light yellow with a few exceptions of orange, pinkish red and diffusible brown, as may be judged from Table 3. The colonies were small, round, entire, slightly raised, translucent to opaque smooth and glistening. The colonies of the larger forms showed a denser central part. Growth characteristics in nutrient broth were more variable than observed in group I. Though the majority of the organisms showed moderate to good uniform turbidity, eight strains formed a thick ring or thin pellicle and seven strains showed poor growth with a viscid sediment (Table 2). The pH range for growth was between 6.0 to 9.0, though a few strains could grow even at pH 4.5. The optimum temperature for all the strains was 25–30° and poor growth was observed at 40°C. Four strains failed to grow above 37°.

Group—III. The organisms belonging to this group were thicker in diameter than organisms of group I and II and often appeared to be oval in shape. They generally appeared to be larger in young cultures when the angular arrangement was more pronounced. Breakage into coccoidal forms occurred at the end of 36 to 72 hours, the coccoidal forms having a tendency to mass together. Cell wall staining showed the presence of two to three septa per cell. Occasionally filament formation was observed, the filaments showing the presence of a number of septa. The arrangement could be observed in some cases to be of the 'parallel adjacent type' (Sundman, 1958). The organisms were Gram positive to a varying degree in young cultures but became distinctly positive in old cultures. Out of twenty-eight strains which belonged to this group, thirteen were nonmotile, ten showed the presence of a single polar flagellum, two showed one to two lateral flagella and the remaining three showed six to eight peritrichous flagella (Table 1).

The pigment varied from light yellow to orange. Colonies were generally round, entire, convex, smooth, glistening, opaque and about 1–2 mm. in diameter. Growth characteristics in nutrient broth were of a variable type; thirteen strains

formed either a thick ring or thin pellicle with the broth moderately turbid and with two strains the broth remained clear. Out of the remaining fifteen strains, nine showed moderate to good growth with uniform turbidity and six showed poor growth with a compact viscid sediment (Table 2). The pH range for the organisms was found to be between 6 to 8.5, except for two strains which could grow even at pH 4.5 and 9.0. The optimum temperature was between 25-30° and the maximum was 40°. Two strains could not grow beyond 37°.

Group IV. The organisms included in this group were quite distinct from the above three groups in that they were thin rods showing a very pronounced angular arrangement and a tendency to the typical 'Chinese letter' formation that is known to characterize *Corynebacterium*. Branching was distinct in young cultures, the organisms breaking up into smaller unbranched forms with age. The ultimate unit of breakage was however not coccoidal as in other groups but coccobacillary. These cultures on cell wall staining did not clearly reveal septation or internal morphology, the entire cell being characterised by excessive granulation. All strains were weakly Gram positive. All strains belonging to this group were nonmotile in young cultures but doubtfully motile to motile when old. All but six strains were polar flagellates (Table 1).

TABLE II
Growth characteristics in nutrient broth

Morphological group and number of strains in each group	Thick ring of pellicle accompanied by uniform turbidity	Thick pellicle on an otherwise clear medium	Uniform moderate turbidity without pellicle	Scant or no turbidity without pellicle but with a viscid sediment
I (13)	2	0	9	2
II (49)	6	2	34	7
III (28)	11	2	9	6
IV (24)	2	0	18	4

Pigmentation was as a rule yellow. The colonies of this group were small, punctiform, round, entire, slightly raised and translucent, becoming slightly granular and opaque with dark centres on aging. With a few strains, filamentous and arborescent type of surface was observed. Growth in nutrient broth was slow at first, becoming moderate by about the fourth day. Most of the strains showed a moderate uniform turbidity, but two strains formed a thin pellicle and four strains formed a compact viscid sediment (Table 2). The pH range was 6-9.0. The optimum temperature was 25-30°, but poor growth was secured upto 40°. Three strains failed to grow at temperatures above 37°.

PHYSIOLOGICAL CHARACTERISTICS

The physiological characteristics exhibited by the strains are presented in Tables 3 to 7.

TABLE III

Physiological characteristics

Physiological Tests	Morphological group and the number of strains in each group			
	I (13)	II (49)	III (28)	IV (24)
1-Methyl Red Test	2	23	8	8
2-Voges Proskauer test	1	12	6	2
3-Production of indole	1	0	2(1)	0
4-Production of hydrogen sulphide	6(4)	14(7)	6(4)	6(3)
5-Production of urease	0	5	7	0
6-Production of catalase	13	47	26	21
7-Production of dehydrogenase	5 (1R; 2PR; 2RO)	18 (6R; 6PR; 6RO)	11 (6R; 2PR; 3RO)	16 (12R; 1PR; 3RO)
8-Production of gas from NaNO_3 under alkaline anaerobiosis	0	2	0	0
9-Reduction of nitrates to nitrites	9	42	22	21
10-Production of ammonia	7	34	16(2)	7(1)
11-Hydrolysis of casein	3	19	9	6
12-Hydrolysis of fat	0	18	7	4
13-Hydrolysis of gelatine	7	25	13	16
14-Hydrolysis of starch	10	27	17	21
15-Decomposition of cellulose	0	0	0	0
16-Changes in litmus milk	4(R); 3(P); 3(RP)	10(R); 28(P); 2(RP); 2(AC)	6(R); 12(P); 4(RP); 1(AC)	3(R); 7(P); 5(RP); 2(AC)
17-Pigmentation on nutrient agar				
(a) None	---	---	---	2
(b) Grayish white to creamy white	2	22	8	4
(c) Yellow	8	15	6	17
(d) Orange	2	4	12	---
(e) Pink	1	3	2	---
(f) Diffusible brown	---	2	---	---

Figures indicate the number of strains showing a positive reaction.

Figures in parenthesis indicate strains giving a late positive reaction.

Changes in litmus milk: R, reduction; P, peptonisation; RP, reduction and peptonisation; AC, acidity and coagulation.

Production of dehydrogenase: R, reduction of methylene blue; RO, reduction and reoxidation of methylene blue; PR, partial reduction.

TABLE IV

Ability to metabolise carbohydrates under aerobic and anaerobic conditions

Morphological group and number of strains in each group	Number of strains that metabolise all or most sugars oxidatively	Number of strains that metabolise all or most sugars fermentatively	Number of strains that metabolise all or most sugars oxidatively and fermentatively.	Number of strains that attack carbohydrates indifferently	Number of strains that are inert or weak in carbohydrate metabolism
I (13)	1	0	1	6	4
II (49)	5	0	4	7	33
III (28)	3	0	4	3	18
IV (24)	12	0	3	3	6

TABLE V
Utilization of organic carbon compounds

Carbon compounds	Morphological group and number of strains in each group			
	I (13)	II (49)	III (28)	IV (24)
L-Asparagine	5	31	15	8
Acetone	7	16	17	11
Aniline	4	24	17	14
Benzene	6	25	18	16
Naphthalene	4	11	9	6
Anthracene	3	22	11	18
Alpha-naphthol	5	17	12	5
Beta-naphthol	0	8	3	5
Phenol	2	16	8	3
o-cresol	0	2	2	0
m-cresol	1	2	4	2
Benzaldehyde	0	6	6	2
Paraffin	0	11	3	0
Caffeine	4	16	16	16
Sodium formate	0	15	11	8
Sodium oxalate	0	0	0	2
Sodium acetate	6	40	22	11
Sodium oleate	8	42	25	16
Sodium propionate	0	3	0	0
Sodium lactate	10	46	26	21
Sodium succinate	9	45	24	18
Sodium citrate	9	29	23	16
Sodium benzoate	8	24	11	0
Sodium salicylate	0	9	0	0
Sodium barbiturate	5	23	15	3
Sodium hippurate	0	0	0	0

Figures indicate the number of strains showing moderate to good growth after incubation at 28-29° for one week.

TABLE VI
Utilization of inorganic nitrogen compounds

Nitrogen compounds	Morphological group and number of strains in each group			
	I (13)	II (49)	III (28)	IV (24)
NaNO ₃	6	23	18	6
(NH ₄) ₂ SO ₄	12	45	23	18
(NH ₄) ₂ HPO ₄	8	24	12	8
NH ₄ NO ₃	9	29	17	16

Figures indicate the number of strains showing moderate to good growth after incubation at 27-29° for one week.

TABLE VII

Utilization of individual amino acids as a source of nitrogen only (N) and as a sole source of carbon and nitrogen (CN)

Morphological group and number of strains tested from each group	dl-Glycine	dl-Alanine	DL-Serine	DL-Norleucine	D-Threonine	L-Leucine	DL-Valine	DL-Isoleucine	DL-Aspartic acid	DL-Glutamic acid	L-Agrinine	DL-Lysine	L-Asparagine	L-Histidine	L-Proline	L-Hydroxyproline	dl-Tryptophane	L-Tyrosine	dl-Methionine	L-Cystine
I (11)	7 4 5 10	2 7 0 6	0 11 1 6	0 5 0 7	5 5 3 9	3 9 3 6	2 10 7 10	7 10 4 10	4 9 0 9	0 5 0 8	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
II (21)	19 17 19 12 14	7 14 7 14	3 16 14 11	0 14 6 20	17 20 17 18 18	19 17 21 14 21	19 11 11	9 3 14 0	8 3 8 0	9 0 9 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
III (10)	10 2 9 9 9	2 10 0 10	2 10 10 10	2 10 3 10	8 10 9 10	8 10 9 10	9 10 9 10	8 8 3 8	0 8 0 8	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
IV (9)	8 0 7 3 7	0 7 0 7	0 8 5 8	5 6 0 9	4 9 7 9	6 9 3 9	6 9 3 9	9 5 9 5	6 6 4 7	0 8 0 8	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0

Figures indicated the number of strains showing moderate to good growth at the end of one week at 27-29°

TABLE VIII

Number of strains exhibiting intermediate physiological characteristics between accepted species

A. Species	<i>A. globiformis</i>	<i>A. globiformis</i> intermediates	<i>A. simplex</i>	<i>A. simplex</i>
Physiological characteristics	Starch hydrolysed; poor growth at 37°	Starch not hydrolysed; poor growth at 37°	Starch hydrolysed; good growth at 37°	Starch not hydrolysed; good growth at 37°
number of strains	8	3	11	14
B. Species	<i>A. pascens</i>	<i>A. pascens</i>	<i>A. simplex</i>	<i>A. simplex</i>
Physiological characteristics	Starch hydrolysed; poor growth at 37°	Starch not hydrolysed; poor growth at 37°	Starch hydrolysed; good growth at 37°	Starch not hydrolysed; good growth at 37°
number of strains	4	0	3	14
C. Species	<i>A. aurescens</i>	<i>A. aurescens</i>	<i>A. ureafaciens</i>	<i>A. ureafaciens</i>
Physiological characteristics	Starch hydrolysed; nitrate reduced	Starch hydrolysed; nitrate not reduced	Starch not hydrolysed; nitrate reduced	Starch not hydrolysed; nitrate not reduced
number of strains	38	7	10	6

DISCUSSION

The group of bacteria under consideration have a striking morphological similarity to *Corynebacterium*, a genus first created by Lehmann and Neumann (1896) to include the *C. diphtheriae* and related parasitic bacteria. Over the decades, however, the boundaries of this genus were widened to include several related forms which nevertheless exhibited a confusing diversity of characters. A situation which permitted the inclusion of animal parasites, soil saprophytes (Kisskalt and Berend, 1918) and plant pathogens (Jensen, 1934; Dowson, 1949) in a single genus, understandably evoked protests.

The genus *Arthrobacter* was first suggested by Conn and Dimmick (1947) to accommodate separately, the saprophytic soil coryneforms having a more varied cell morphology, weaker Gram reaction and acid production and simpler nutritive requirements than the parasitic corynebacteria. The contributions of Lochhead and Burton in recent years (1953, 1955, 1956) have however indicated that several saprophytic coryneforms do have complex growth requirements. While the suggested type species *A. globiformis* was a non-motile strain, several independent investigators have recorded both motility and flagellation in the group (reviewed by Khambata and Bhat., 1955). In the recent edition of Bergy's Manual (Breed *et al.*, 1957), the genus *Arthrobacter* has been recognised. The criteria for its separation and characterisation are its saprophytic nature, morphology, primary habitat in soil, inability to decompose cellulose and usual lack of motility. Since as many as 57 out of the 114 strains in our collection were motile, not much weight can be attached to the lack of this property in characterising the genus. Neither are the morphological distinctions of the group sufficiently exclusive to be useful. It would thus appear that the present basis for the separation of the saprophytic *Arthrobacter* rests largely on its habitat. In addition, our studies have indicated that the genus may be physiologically characterized by an inability to produce

indole, an ability to produce catalase and by a general weakness for carbohydrate metabolism which when it occurs may range from pure oxidative to partly or purely fermentative.

While the accordance of generic status to the soil coryneforms has much to commend itself, some of the criteria that have been used for the further delineation of the genus into species have necessary limitations. Our studies have indicated that the ability to hydrolyse starch, to reduce nitrate and to grow at 37° are characteristics that are too variable to be accorded the importance that is at present given to them (see table 8). On the other hand, characteristics such as chromogenicity (only the presence or absence of pigment; variations in type of pigment occur) and the ability to utilise inorganic nitrogen appear to be sufficiently stable to be useful in a system of classification.

The comparative studies made on the morphology of the strains after staining the cell wall and septa, have confirmed the suspicion that they do not constitute a morphologically homogeneous group. This fact in itself should suggest the use of morphology as an important criterion in the classification of the genus. The simplicity of Robinow's cell wall staining technique and the ease with which it may be applied to large collections renders it as a test that may be routinely used. Indeed, this test deserves to be practised in all studies on bacterial systematics. On the basis of morphology alone, it is tentatively proposed that the *Arthrobacter* may be divided into four morphological groups. Within the limits of the experience gained in our laboratory, it has been observed that the morphological traits described are sufficiently stable under the specified conditions to permit of such a primary morphological classification. It will be further observed that some physiological characteristics such as the utilization of benzoate, paraffin and some amino acids show a correlation with the morphological differentiation. Each morphological group would lend itself to further classification on the basis of physiology, presenting a scheme analogous to the relatively satisfying picture presented by the genus *Bacillus* (Breed *et al.*, 1957). Before accepting such a scheme of classification, two lines of investigation would be in order, (a) comparative studies on a larger collection of saprophytic coryneforms, (b) studies on morphological variations intended to determine whether the characteristics to be used lie sufficiently beyond the limits of variability of a single species. It is to be hoped that the present study would stimulate investigations on these aspects of the systematics of an interesting group of bacteria.

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STUDIES ON THE TETRAPLOIDS OF SIX VARIETIES OF GREEN GRAM

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ABSTRACT

1. Exposure of the seeds to six different doses of colchicine gave very few colchipooids, while practically all the doses in the apical bud treatments were successful. The affected plants were mostly mixoploids, which gave tetraploid and diploid progenies.

2. The tetraploids germinated 2-3 days later and 80-85 per cent of the seeds germinated. They grew slowly and at harvest two varieties remained smaller, two attained the same size and the other two exceeded the diploid in height. Three varieties produced fewer branches. The early flowering varieties flowered about a week later, but the flowering was prolonged in all.

3. The leaves were smaller, except in one variety, thicker due to increased size of palisade and spongy layers; darker green in colour having more chloroplastids per cell; and the lobing was less pronounced in the pinnatisect type.

4. Gigantism in all floral parts were seen, the increase in the size of the standard being most conspicuous; more flowers opened in the first inflorescence but fewer fruits were set, one variety failed to produce any fruit. The fruits were smaller, and the number of seeds per fruit were fewer. The seeds were, however, larger and heavier. Seed yield per plant was reduced and ranged from about 10 to 50 per cent of the respective diploids.

5. About 20 per cent of the pollen were empty, the others were of variable size and about 10 per cent were rectangular with four germ pores.

6. The tetraploids had 44 chromosomes, synapsing mostly in twos, average number of quadrivalents being 1.6 to 1.8 per cell, the trivalents and univalents were still fewer. Other irregularities such as precocious movement, unequal separation and laggards did not exceed 15 per cent of the cells examined.

7. Hybridization reciprocally with the diploids failed.

8. The F_2 progenies of the two inter-tetraploid crosses were highly variable and some plants did not set any fruit at all, but about 15 per cent of the plants exceeded the diploid mean in yield and appear to be promising as a source for increasing tetraploid yield.

INTRODUCTION

Green gram, *Phaseolus aureus* Roxb., syn *P. radiatus* L. is an important legume of South East Asia. It is grown for its nutritious seed and also as a fodder crop. Tetraploid of a variety of green gram was produced by Kumar and Abraham (1942) using colchicine. According to Kumar (1945) the tetraploid was late in flowering, more susceptible to disease and extremely poor yielding, and no improvement was noticed even though the tetraploids were selected through three successive generations. They had reduced number of branches, flowers, pods and seeds per pod. The size of the petal and breadth of the fruit were, however, bigger.

With the advent of the colchicine technique, polyploids have been produced in a large number of plants, but very few of them could be used immediately. Differential response observed among the varieties due to tetraploidy in morphological and physiological characters by Muntzing (1948) in barley, Kuckuck and Levan (1951) in linseed and by many others, drew attention of the breeders to the importance of broadbasing the tetraploid breeding programme by including a large number of diploids to start with. It has also been shown that by selection among the progenies of the tetraploids, considerable improvement could be made. To date, greater successes in polyploid breeding have been obtained in cross fertilized

plants owing to the heterozygosity of the material, red clover (Levan 1942), steel rye (Muntzing 1951) being outstanding examples. Similarly recombination breeding has given promising results in autogamous plants as in flax (Kukh 1943), barley (Muntzing 1948) etc.

A project for inducing tetraploidy in several varieties of kharif (summer) and rabi (autumn) green gram was undertaken. An evaluation of the dosage in inducing colchiploids, the effects of tetraploidy on different morphological characters, fertility, karyology and the results of hybridization are given in this paper.

MATERIALS AND METHODS

Four kharif varieties—NP 28, EB 6, 4441-D1, Chinimung and two rabi varieties—BR 3 and Sonamung from our genetic stock (Sen and Ghosh 1959) were selected. They were grown and treated in their respective seasons.

Soaked seeds were treated in aqueous solution of colchicine after removing the seed coat, in 0.01, 0.25 and 0.5 per cent solutions for $\frac{1}{2}$ to 24 hours, washed in water and sprouted in petri-dishes. The seedlings were raised in wooden boxes and then transplanted in field. In the seedling treatment, the growing tips of a week old seedlings between the first pair of leaves were treated through cotton plug, covering the seedling with small pots to check evaporation. The concentrations of colchicine used were 0.25 and 0.5 per cent and the duration varied from 1-6 hours and repeated for 3 days in a few cases.

The effectiveness of the different treatments and the dosages have been compared. The heights at harvest, number of nodes and branches, size and thickness of leaves, stomatal size and frequency, flowering time and flower size, pollen size and fertility, number of fruits per plant and the number of seeds per fruit and yield per plant were recorded and given in Tables 1 and 2.

Meiotic studies were made in pollen mother cells from flower buds, fixed in acetic alcohol (1:3), smeared in acetocarmine after mordanting in 4 per cent iron alum. For mitotic chromosome preparations the freshly cut root tips were treated in saturated solution of paradichlorobenzene for $1\frac{1}{2}$ to 2 hours in room temperature, washed, gently heated for 3-5 minutes in a mixture of 2 per cent aceto-orcein 9 parts and 1 part N HCl and stained in 1 per cent aceto-orcein. Photomicrographs were taken at $\times 250$ and printed at $\times 1300$ and $\times 1500$.

For hybridization, flower buds due to open the next day were emasculated in the evening and pollinated in the next morning. Oil paper bags were used.

OBSERVATIONS

Comparative Success of Seed and Seedling Treatments : In all the seed treatments swelling of sprouted roots and hypocotyl were observed. The root growth was checked and recovery took place only in lower doses. The surviving seedlings grew very slowly, the two fleshy cotyledons persisted for much longer duration, the first pair of leaves were short and thick plaited along the midrib and incurved. The second leaf had small rough leaflets, which were variously modified. Even with the maximum cultural care, most of the seedlings died and the surviving ones grew slowly, remained shorter and flowered later. Each inflorescence was checked for bigger sized flowers and pollen sterility. Of the 250 seeds treated in 6 doses 150 plants survived but only three colchiploids were obtained.

In the seedling treatments, the primary effect was to check the growth of the apical bud more or less proportional to the dose. The first pair of leaves below the apical bud became larger, thicker and darker green in colour. The treated plants grew slowly and remained smaller and the first three leaves showed

TABLE I

Comparative effects of tetraploidy on leaf size and thickness, height at harvest, total nodes and branches, flowering time in days, standard width and pollen sterility in the different varieties

Characters	Ploidy	NP 28	EB 6	4441-D1	Chininung	BR 3	Sonamung
Leaf length (cm)	2n 4n	— —	12.2±0.30 9.0±0.31	12.3±0.47 9.6±0.55	11.7±0.33 9.7*	6.1±0.14 6.8±0.15	5.3±0.20 3.9±0.26
Leaf width (cm)	2n 4n	— —	11.0±0.14 8.9±0.39	9.2±0.28 8.9±0.53	9.5±0.28 9.7*	5.0±0.15 6.7±0.14	4.3±0.16 4.2±1.18
Leaf thickness (in microns)	2n 4n	— —	278.8±0.63 353.1±0.43	286.2±0.14 343.1±0.39	214.9±0.15 312.7*	280.8±0.82 360.6±0.94	267.9±0.75 268.6±0.60
Height at harvest (cm)	2n 4n	39.6±2.12 55.0±2.81	38.1±1.21 45.1±2.91	40.8±1.23 39.4±2.33	35.6±1.25 21.5*	38.6±2.30 39.0±2.94	27.6±1.06 20.2±0.60
Total nodes on main stem	2n 4n	14.8±0.49 16.1±1.31	13.0±0.14 14.3±0.32	14.3±0.34 13.9±0.48	12.8±0.32 13.5*	10.4±0.22 10.4±0.26	9.5±0.30 8.8±0.86
Total branches	2n 4n	3.9±0.27 3.7±0.21	3.7±0.15 3.0±0.44	5.3±0.32 4.2±0.42	4.2±0.23 2.5*	2.9±0.09 1.7±0.21	2.8±0.19 1.0±0.25
Flowering time (days)	2n 4n	46.0±0.59 46.5±1.03	37.2±1.55 46.6±1.08	45.6±0.86 46.1±1.65	46.6±0.71 46.0*	29.2±0.28 33.1±0.56	26.7±0.53 31.6±0.76
Standard width (cm)	2n 4n	1.5±0.03 1.9±0.01	1.7±0.01 2.0±0.01	1.7±0.02 2.0±0.01	1.7±0.02 2.0*	1.6±0.02 1.9±0.03	1.4±0.01 1.9±0.03
Pollen sterility (percent)	2n 4n	3.1±0.09 22.7±	3.2±0.01 28.2±0.67	3.0±0.02 26.2±1.19	3.5±0.12 24.8*	3.1±0.10 17.4±0.51	3.4±0.09 20.8±0.68

* Average of 3 plants

TABLE II
*Comparative effects of tetraploidy on the pollen sterility, number of flowers and fruits on the first inflorescence,
 fruit size, number of seeds per fruit, seed size, and yield per plant*

Characters	Ploidy	NT-2s	EB 6	4441-D1	Chinnung	BR 3	Sonamung
Pollen sterility	2n 4n	3.1 ± 0.09 22.7 ± 0.73	3.2 ± 0.11 28.2 ± 0.67	3.0 ± 0.17 26.2 ± 1.19	3.3 ± 0.12 24.8*	3.1 ± 0.10 17.4 ± 0.52	3.4 ± 0.09 20.8 ± 0.68
Number of flowers on 1st inflorescence	2n 4n	8.8 ± 0.47 13.4 ± 0.91	6.4 ± 0.91 13.3 ± 0.66	6.6 ± 0.17 13.9 ± 0.79	5.2 ± 0.21 13.5*	4.5 ± 0.21 8.4 ± 0.88	4.5 ± 0.30 8.0 ± 0.48
Number of fruits on 1st inflorescence	2n 4n	7.4 ± 0.47 6.5 ± 1.00	4.0 ± 0.31 2.0 ± 0.14	4.7 ± 0.54 1.7 ± 0.40	4.1 ± 0.23 —	3.2 ± 0.38 2.2 ± 0.38	2.8 ± 0.32 1.1 ± 0.30
Fruit size (cm)	2n 4n	7.0 ± 0.15 5.0 ± 0.24	8.8 ± 0.12 5.2 ± 0.30	7.3 ± 0.16 4.7 ± 0.29	8.2 ± 0.21 —	5.4 ± 0.25 4.2 ± 0.25	5.5 ± 0.27 3.4 ± 0.39
Number of seeds per fruit	2n 4n	12.3 ± 0.42 5.8 ± 0.53	11.8 ± 0.31 3.4 ± 0.33	11.4 ± 0.94 4.6 ± 0.43	11.5 ± 0.50 —	10.6 ± 0.23 4.8 ± 0.54	7.4 ± 0.45 2.9 ± 0.49
Seed size (mm)	2n 4n	2.9 ± 0.06 3.5 ± 0.15	3.8 ± 0.05 4.7 ± 0.09	3.6 ± 0.06 4.1 ± 0.11	3.8 ± 0.08 —	3.2 ± 0.05 4.2 ± 0.09	3.4 ± 0.07 3.9 ± 0.11
Weight of 100 seeds (gms)	2n 4n	2.0 ± 0.06 2.6 ± 0.12	5.2 ± 0.08 5.7 ± 0.15	3.3 ± 0.02 4.1 ± 0.14	5.1 ± 0.02 —	2.8 ± 0.06 4.1 ± 0.16	2.0 ± 0.05 2.8 ± 0.13
Yield per plant (gms)	2n 4n	17.9 ± 2.15 8.6 ± 1.55	16.6 ± 1.03 2.1 ± 0.49	31.2 ± 0.06 3.5 ± 0.44	17.4 ± 1.93 —	14.8 ± 0.25 3.4 ± 0.13	12.5 ± 0.08 1.0 ± 0.05

* Average of 3 plants

different types of deformities. Practically all the treatments used gave colchipooids. The lowest dose 0.25 per cent—1 hour gave the minimum number of colchipooids, the dose repeated for three days increased the frequency more or less proportional to the treatment for 3 hours at a time. The 0.25 per cent—3 hours repeated for 3 days gave about 35 per cent colchipooids, the maximum number among all the treatments, 0.5 per cent—3 hours for 1 day was the next best.

The progenies of these mixoploid plants, even when raised from diagonalised tetraploid sectors always had some diploids. In the lines, however, most of the tetraploids could be identified from their general appearance.

Effects of Tetraploidy: In general, the tetraploids germinated 2-3 days later and about 80-85 per cent of the seeds germinated. Laboratory test showed that some of the tetraploid seeds failed to imbibe water unless the seed coat was mechanically ruptured, viability of all of them being good. The hypocotyl of the tetraploids were a bit thicker and the first pair of leaves thicker and darker green in colour.

The tetraploids of green gram can be easily identified from the appearance of their leaves, which are fleshier and darker green. Fleshiness is due to increased size of the palisade and spongy layers and the darker green colour for more chloroplastids per cell. In size the leaves were reduced, except in the variety BR 3 and the lobing was less pronounced in the pinnatisect type 4441-D1. Stomatal size increased and the frequency of stomata per unit area was reduced.

The tetraploids in general grew slowly. The height at harvest was less in the varieties Chinimung and Sonamung, more or less the same in 4441-D1 and BR 3 and increased in EB 6 and NP 28. In the last two varieties the total number of nodes also was more, while in the others no difference was observed. The tetraploids of the varieties Chinimung, BR 3 and Sonamung produced fewer branches, but in the other three varieties the number of branches were more or less the same as in the diploids.

The mean flowering time in days after sowing in half the varieties, where the flowerings in the diploids were comparatively earlier (26 to 37 days) was later in the tetraploids. In the other three varieties, where the flowering was delayed till 45th day, the tetraploids flowered simultaneously with the diploids.

The tetraploid flowers were conspicuously larger and the ventral side of flower buds split open. In fact, the tetraploid sectors in the colchipooid generation could be detected from the flower size and the appearance of the buds. Practically all the floral parts were increased in size, the increase in the standard width being most conspicuous.

The number of total inflorescence on the main axis was not affected, but the inflorescence axes were shorter and stouter. Though the number of flower buds on the first inflorescence were more or less similar, many more flowers opened in the tetraploids. The flowering was also much prolonged in the tetraploids.

The tetraploids had more flowers per inflorescence, but the number of fruits per inflorescence was fewer. However, even a single fruit could not be obtained in the three plants of the variety Chinimung. Pollen sterility was about 20-30 per cent and the pollen sizes were highly variable. In addition to the general increase in size of the majority of the pollen, a conspicuous feature of the tetraploids was the presence of squarish pollen with four germ pores, not seen in the diploids. About 10 per cent of pollen grains were of this type. The tetraploid ovules were slightly bigger, but the number was not affected. While most of the ovules matured to seeds in the diploid, 50-70 per cent of the tetraploid ovules aborted at different stages of growth.

The tetraploid fruits were shorter and thicker. The number of seeds per fruit was fewer, but the seeds were bigger with thicker seed coat. Weight of 100

seeds were more, but the total yield was less being about 10 per cent of the diploid in Sonamung to about 50 per cent in NP 28. The tetraploids of the three varieties, viz., EB 6, 4441-D1 and Sonamung were susceptible to brown spot fungus disease which considerably affected their growth and yield.

Meiosis in all the diploids were regular, showing eleven bivalents at diakinesis and first metaphase. In the tetraploids, bivalents were most common, though varying number of quadrivalents and a few trivalents and univalents were seen. Frequencies of the different types of configuration in the three varieties are given in Table 3, counted from 20 clear diakinesis and metaphase plates.

TABLE III
Frequencies of different configurations at meiosis

Varieties	IV-valent		III-valent		II-valent		I-valent	
	range	mean	range	mean	range	mean	range	mean
NP 28	0-4	1.68	0-1	0.15	14-22	18.25	0-2	0.21
BR 3	0-4	1.78	0-2	0.20	14-22	18.84	0-3	0.45
4441-D1	0-4	1.66	0-2	0.43	12-22	17.28	0-2	0.66

Precocious movement of one or two chromosomes in metaphase and lagging of one or two chromosomes were seen in about 10 per cent of the cells examined. Though majority of the clear plates showed 22-22 chromosomal distribution, several plates with unequal distribution were seen in about 10 to 15 per cent of the cells. Similar unequal separation and laggards were common in the second division. In addition to the tetrads, pentads and hexads were seen, the frequency of the polyspored cells was about 10 to 15 per cent.

The mitotic chromosomes were small, mostly with submedian primary constriction. There were twenty-two chromosomes in the diploids and forty-four chromosomes in the tetraploids.

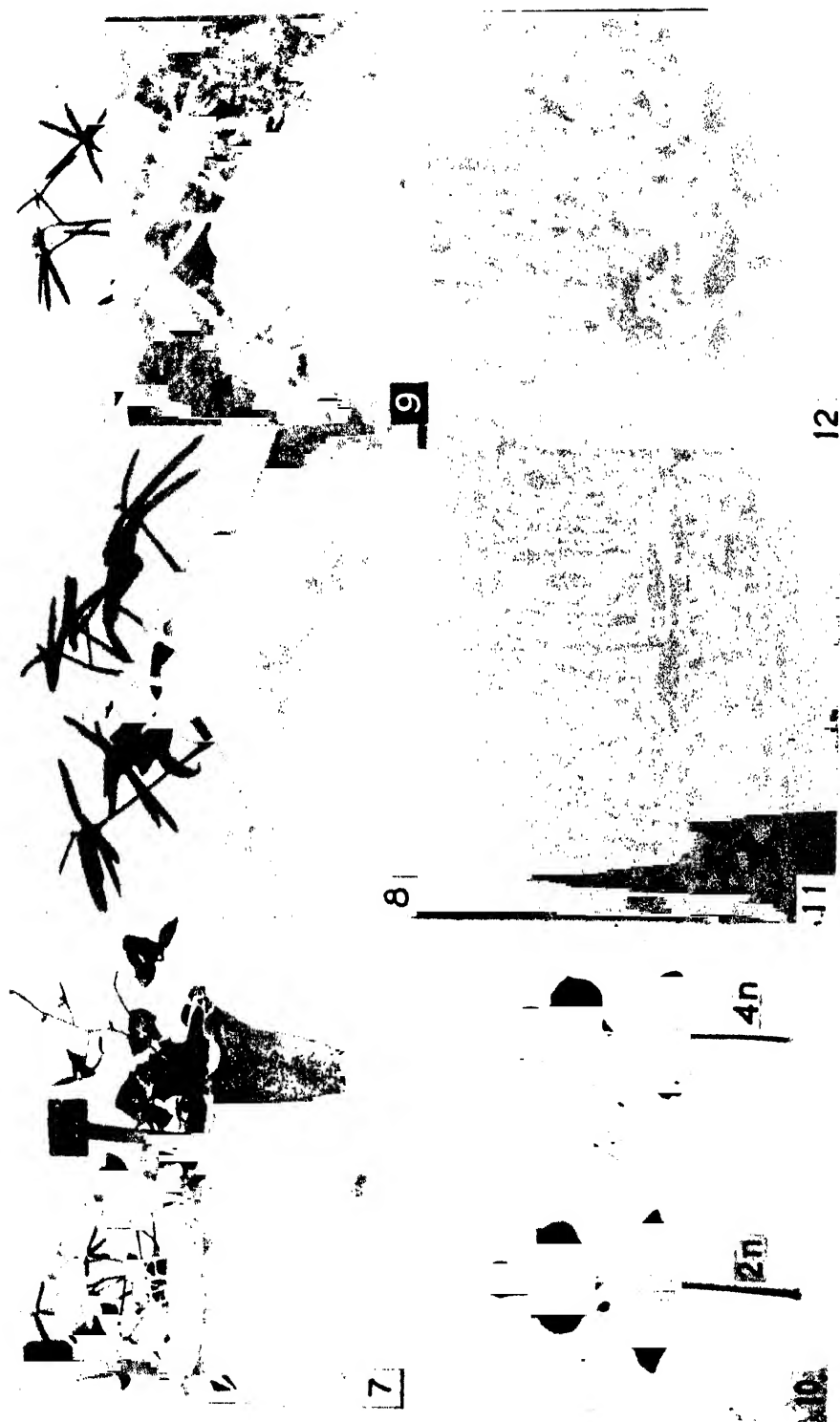
Hybridization : To study the extent of isolation of the tetraploids from the diploids and also to produce triploids, if possible, attempts were made to cross diploids and tetraploids reciprocally in the three varieties NP 28, EB 6 and 4441-D1. It was observed that when the diploids were used as female parent no fruit was set, but when the tetraploids were used as the female parent young fruits developed in about 20 per cent of the crosses. Along with the fruits the ovules grew, but most of the fruits were shed off, when they were about 3 to 4 cm. long.

The F_1 plants of the cross EB 6 \times 4441-D1 and EB 6 \times Chinimung gave more fruits and seeds than either of the parents, and that of the cross NP 28 \times 4441-D1 the yield was intermediate between the two parents.

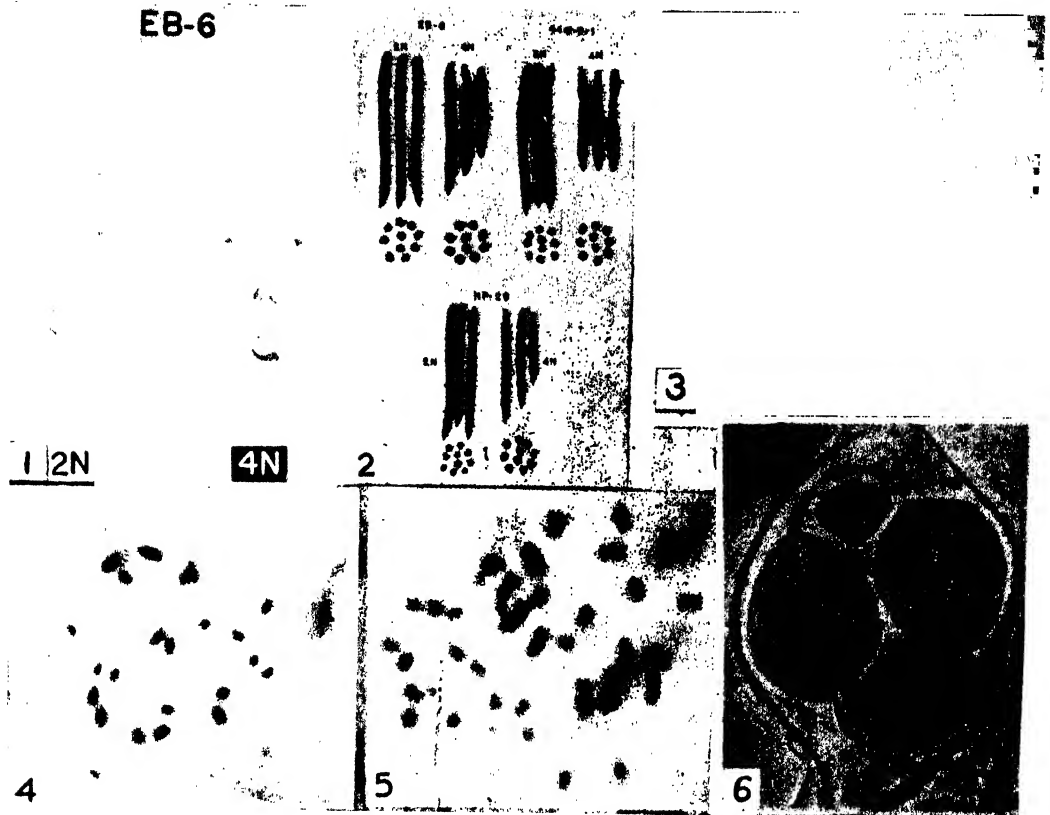
The F_2 plants showed a wide range in yield, but as a portion of the field was water logged and the plants did not grow well there, no record of yield data was taken. But, out of 74 F_2 plants of the cross NP 28 \times 4441-D1, 16.2 per cent bore no fruit, 64.86 per cent had few fruits, and 18.92 per cent plants had a good number of fruits, some of which even surpassed the diploids. Out of the 52 F_2 plants of the cross EB 6 \times 4441-D1, 15.6 per cent plants also surpassed the diploids in yield. How far the environmental factors are responsible for good yield in this 15 to 18 per cent of the heterozygous tetraploids and whether by selection from these plants, yield of the tetraploids can be increased cannot be said now, but the general appearance of the plants is encouraging.



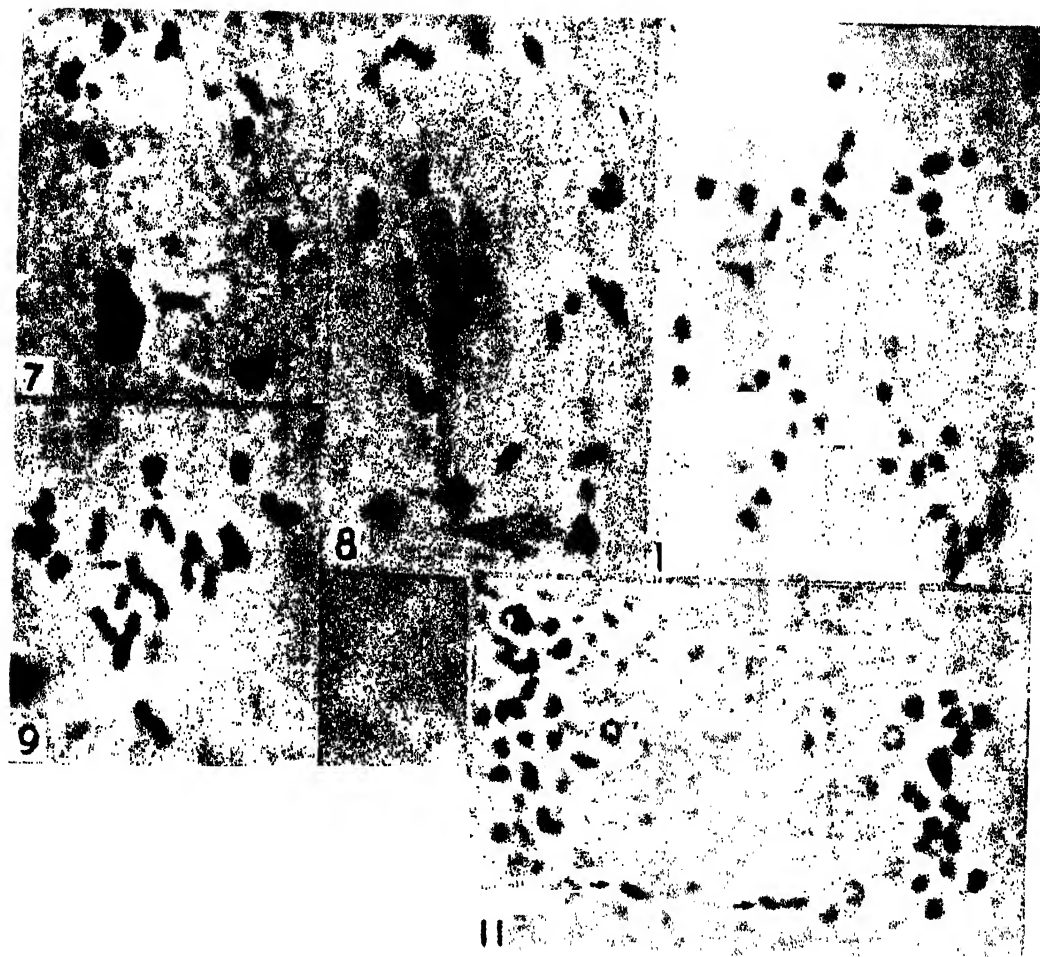
FIGS. 1.—Diploid and tetraploid branches in a treated plant. 2 and 3. Diploid and tetraploid plants of E.B. 6. 4 and 5. Diploid and tetraploid plants of N.P. 28. 6. Diploid and tetraploid plants of 441 D 1.



FIGS. 7. Diploid and tetraploid plants of Chininung; 8, and 9. Selection from F_2 generation of the cross N.P. 28 (4n) \times 4441-D 1 (4n).
10. Effect of triploidy on leaf lobing in 4441-D 1, 11 and 12. Sections of diploid and tetraploid leaves of N.P. 28.



FIGS. 1. Floral parts of diploid and tetraploid of E.B. 6. 2. Fruits and seeds of diploids and tetraploids of E.B. 6, 4441-D 1 and N.P. 28. 3. Pollens of tetraploid showing squarish type. 4 and 5. Somatic chromosomes of diploid and tetraploid E.B. 6. 6. Tetrad with micronuclei in tetraploid.



FIGS. 7 and 8. Diakinesis of diploid and tetraploid EB 6. 9. Metaphase I of tetraploid. 10. Anaphase I of tetraploid. 11. Anaphase I of tetraploid showing laggards.

DISCUSSION

Attempts to induce tetraploidy in six varieties of green gram through seed treatment were practically unsuccessful, as reported earlier by Kumar and Abraham (1942), even though a much wider range of colchicine concentrations and periods of treatment were tried. Though there are some examples of success through seed treatment in legumes as in gram (Ramanujam and Joshi 1941) and in *Trifolium pratense* (Bragdo 1955), it is not a suitable method for inducing tetraploidy in pulses (Sen and Chedda 1958, Bhowal 1958, Vidyabhusan 1959). The failure of the swelled tap root to grow and the nondevelopment of the lateral roots are the common causes of failure of seed treatment in green gram.

On the other hand, all the seedling treatments with different concentrations and exposure periods, gave one or more colchiploids. It seems that treatment of the apical buds of the seedling is a suitable method for inducing tetraploidy in large seeded legumes as has been observed by Straub (1940) in pea, Kumar and Abraham (1942) in green gram, Srivastava (1955) in six varieties of gram, Sen and Chedda (1958) in black gram, Bhowal (1958) in cowpea, Vidyabhusan (1959) in cluster bean, horsegram and soybean, and Sen and Marimuthu (1950) in broad bean.

As expected from the treatment of a multicellular apical growing point, chimeras of diploid and polyploid sectors were produced in most of the affected plants. Tetraploid plants could only be recovered from the next generation.

Considerable varietal difference in the effect of tetraploidy on the six varieties of green gram on several characters was seen. The fleshy dark green leaves of the tetraploids were smaller in the varieties EB 6, 4441-D1 and Sonamung, but had the same width though shorter in Chinimung and was slightly larger in BR 3. The increase in thickness was insignificant in Sonamung, but was appreciably more in all other varieties. Derman (1940) concluded that as chromosome doubling affects the size of plant parts through the effects on cell size and cell numbers making up the part, the tetraploid parts will be larger if with the increase in cell size the cell number is not reduced. As practically all cells of the leaves of the tetraploids were larger, the reduction in size in some of the varieties is likely to be due to the decrease in cell number making up the leaves.

The tetraploid plants in all the varieties grew slowly, a general characteristic of the autotetraploids. The slower rate of growth has been attributed to several factors like reduced rate of cell division, and smaller amount of growth hormone present etc. The height at harvest was less in Chinimung and Sonamung, more or less similar in 4441-D1 and BR 3 but more in EB 6 and NP 28. The total number of nodes on the main stem was more in NP 28 and practically unaffected in the other varieties. The number of branches were unaffected in NP 28, EB 6 and 4441-D1, but was reduced in Chinimung, BR 3 and Sonamung.

The general delay in flowering due to tetraploidy was seen in the three early flowering varieties, but not in the other three where the diploids flowered later. Giganticism in practically all the floral parts of the tetraploids, the organs of limited growth, was very conspicuous. The tetraploids can be identified from their flower size alone. Though the number of flower buds per inflorescence axis was more or less the same, many more flowers opened in the tetraploids and the flowering period was considerably prolonged.

Compared to the autotetraploids in general, the percentage of the empty pollen was not very high in green gram tetraploids. The pollen grains were larger, variable in size and about ten per cent were rectangular in shape with four germ pores, the diploid pollen being triangular with three germ pores. The number of ovules was unaffected, but a large number of them degenerate at different stages of growth. Fruit setting was reduced and also the number of seeds per fruit. The var.

Chinimung failed to set any fruit, and the var. NP 28 had the maximum number of fruits and seeds per fruit. The size of the seeds and weight of 100 seeds increased in all the varieties, but could not compensate for the reduced fruit number, thus much lowered seed yields were obtained. The yield was less than ten per cent of the diploids in Sonamung and about fifty per cent in NP 28.

The number of quadrivalents in the tetraploids of green gram was quite low with an average of 1.6 to 1.8 per cell in the different varieties; the trivalents and univalents were still fewer. Other irregularities, such as precocious movement, unequal separation and laggards did not exceed 15 per cent of the cells examined. Micronuclei and polypores were seen in 10 to 15 per cent of the tetrads. Irregularities in meiosis of the autotetraploids, according to Darlington (1937), arise mostly from the formation of multivalents during synapsis. Kostoff (1940) found that the autotetraploids of species having small chromosomes tend to show fewer irregularities because of reduced multivalent formation due to lower chiasma frequency. The green gram chromosomes are small and thus pairing in twos is expected at the tetraploid level and reduced meiotic abnormalities.

Muntzing (1936) cited some examples of autotetraploids with small chromosomes and bivalent synapsis that are quite sterile and others in spite of multivalent association are highly fertile. Though meiotic irregularities play a big role in bringing about sterility in the autotetraploids, the more plausible explanation for the sterility is due to the upset of the genetic balance brought into the diploids of the cultivated plants through selection for generations. Parthasarathy and Rajan (1953) suggested that the genetic unbalance due to chromosome doubling may be toned up by suitable breeding methods and they succeeded in improving the fertility in the autotetraploids of *Brassica campestris* var *toria* by mass, pedigree selection. Selection alone in cross fertilized plants and recombination breeding in autogamous plants have given encouraging results in improving fertility of several tetraploid plants. In green gram too, Sen and Murthy (1960) found considerable increase in the size of the leaves, height, number of pods per plant, in the autotetraploids, besides increased protein content due to tetraploidy. How far hybridization and selection are going to increase the yield is to be seen and some of the F_2 plants appear to be promising.

ACKNOWLEDGEMENTS

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STUDIES IN THE PROTEACEAE

1. TRIBE PERSOONIEAE

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ABSTRACT

Morphological floral anatomical, embryological and cytological (where fresh material was available) studies have been made in 6 genera and 25 species of the tribe Persoonieae and the data have been made use of in discussing the inter-relationships among the genera and the evolutionary trends within the tribe.

Bellendene ($n = 5$) and *Persoonia* ($n = 7$) are the most primitive and the only diploid genera in the family. The remaining genera (*Symphyonema* $n = 10$; *Cenarrhenes*, $n = 14$; and *Brabeium* $n = 14$) seem to be tetraploids on bases 5 and 7 respectively while *Agastachys* ($n = 13$) seems to be a hypoploid.

The close similarity between the Australian and extra-Australian Persoonieae points to a common ancestry for them. Due to species concentration and presence of the two diploid genera in East Australia, the latter is considered to be the probable centre of origin of the tribe wherefrom ancestral stocks seem to have migrated to other land masses.

INTRODUCTION

The family Proteaceae comprises 63 genera and about 1,400 species of trees and shrubs, with exstipulate, simple or compound, alternate or sometimes opposite or whorled leaves. Many species show xeromorphic features. The flowers occur singly or in groups in the axils of bracts in the subfamily Persoonioideae, in pairs in the Grevilloideae, and form racemose inflorescences. They are hypogynous, monochlamydeous, 4-merous and regular or zygomorphic. Though they are usually hermaphrodite, partial male sterility, gynodioecy and dioecy are noticed in a few genera. There are four valvate tepals which are sometimes petaloid and connate. These are antero-posterior in all Persoonioideae and the relatively primitive Grevilloideae, but diagonal to the bract in the more advanced genera of the latter. The four stamens are antetepalous, 4-locular, epiphyllous and introrse, except in Conospermeae in which they are 2-locular and extrorse. In *Stirlingia* and Conospermeae, the anther loculi are connate. In several members there is a hypogynous nectary with 4 free alternitepalous lobes; these are sometimes fused into a cup and in the more advanced genera, the nectary becomes zygomorphic due to the suppression of one or two anterior lobes. The pistil is monocarpellary and stipitate or sessile; the style is either straight with simple, terminal stigma or curved with a lateral stigma situated at the middle of a discoid pollen collecting apparatus. The ovary bears either 4 or more ovules on marginal placenta, or two or one pendulous, lateral or basal ovules, the micropyle of which faces the base of the loculus. The fruit is indehiscent in the Persoonioideae, being a nut, drupe or samara; in the Grevilloideae it is usually a follicle with winged seeds. The seeds are mostly non-endospermic.

The large majority of the living Proteaceae are confined to the southern hemisphere; species of *Protea* and *Faurea* extend into temperate Africa. *Helicia* is the only genus of which a few species extend as far north as South Japan (Map I).

Robert Brown (1810) divided the Proteaceae into two sub-families, the Nucleamentaceae and the Folliculares; he divided the former into 4 tribes and the latter into 3. Bentham (1870) accepted this classification since it was also in keeping with the geographic distribution of the family : the first sub-family occurs in S. Africa and second in S. America and both sub-families occur in Australia. Engler (in Engler and Prantl, 1894) while accepting Brown's classification in principle, altered the names of the sub-families into Persoonioideae and Grevilloideae respectively. Engler's views regarding evolution within the family are represented schematically in Fig. 1.

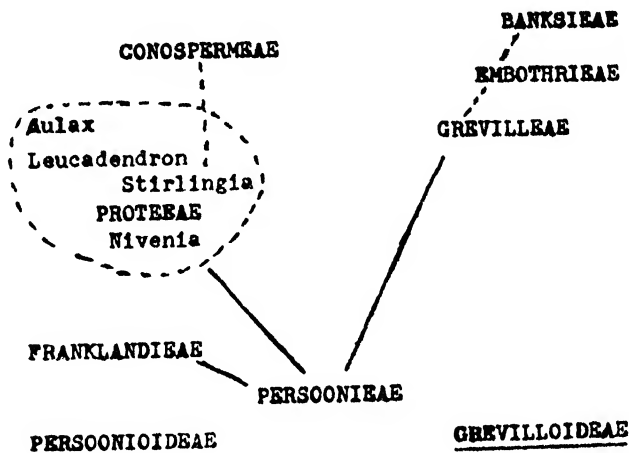


FIG. 1.—Evolution within Proteaceae according to the views of Engler.

Recently Sleumer (1954, 1955a, 1955b) published some taxonomic accounts in which he redefined the limits of some genera of old and new world Proteaceae. He accepted, however, the classification of Engler and made no attempt at redefining the tribes. About 150 species have been added to the Australian Proteaceae since Bentham's (1870) account (Mr. J. H. Willis of Melbourne Herbarium in a personal communication) and new species have been reported also from America (Jose, 1950). The following genera have been added since Engler's publication : *Hicksbeachia* (Mueller, 1883), *Musgravea* (Mueller, 1890) *Hollandaea* (Mueller, 1899), *Placospermum* (White and Francis, 1923), *Austromuelleria* (White, 1930), *Opistholepis* (Smith, 1952) and *Heliciopsis* (Sleumer, 1955b). The systematic position of *Musgravea* and *Austromuelleria* has not been fixed by their respective authors.

There is much diversity of opinion regarding the taxonomic position of the Proteaceae. Bentham and Hooker (1862–1883) include it in the series Daphnales of the Monochlamydeae along with the families Lauraceae, Thymelaeaceae, Elaeagnaceae and Penaeaceae. Engler (1894), Hutchinson (1926), Rendle (1952) and Lawrence (1955) place it by itself in the order Proteales. Lawrence (l.c.) summarises the opinions of the various taxonomists regarding the phylogeny and affinities of the family as follows : "Hallier accepted Engler's view of primitiveness of the order and considered it derived from his Proberberidaceae. Bessey expressed doubt as to its relationship and placed it in the Sapindales as phylogenetically more advanced than the amentiferous families also assigned to the Rosales. Rendle, following Engler, placed the order between the Urticales and Sapindales noting that it was difficult to associate it phylogenetically with other orders. Hutchinson considered it a terminal taxon derived from stocks ancestral to the Thymelaeaceae".

Lawrence himself is of the opinion that the order is not basically primitive though it cannot yet be closely related to any existing order and concludes : "these views are divergent and are evidence of the need of much further study on the phylogeny of the order".

PREVIOUS WORK

Cretaceous and tertiary fossils, supposed to belong to the Proteaceae, have been reported from different parts of the world including Greenland in the north and Grahamland in the south. Kausik (1943) reviewed the previous literature on the subject. Since then, Simpson has reported pollen of *Fauria*, *Petrophila* and *Laubertia* from the Tertiary of Scotland (Walton, 1953). Cookson (1950, 1953, 1956), Cookson and Duigan (1950), Cookson and Pike (1954) and Pike (1953) have described proteaceous pollen and fruits of *Banksia* from Tertiary of Australia. There is still much controversy regarding the occurrence of fossil Proteaceae in the northern hemisphere. This topic will be considered later in an article dealing with the origin of the family.

Chromosome numbers have been determined in 8 African genera by de Vos and about 15 Australian genera by Lancaster, and Blackwood (cf. Brock, 1954, and Darlington and Wylie, 1955).

Chattaway (1948a, 1948b) has studied the wood anatomy of about 30 genera of the family. The wood of *Banksia* and *Dryandra* which form a very natural tribe, the Banksieae, is not only distinctive from that of other tribes of the family but unique in showing radially aligned vascular tissue in the rays. Chattaway opines (in a personal communication) that in other tribes it is difficult to describe the wood as primitive or evolved or distinctive.

Kausik (1938b, 1940a, 1941) studied the floral anatomy of *Macadamia ternifolia* and *Grevillea robusta* and came to the conclusion that the perianth in the family represents the calyx and that the nectary is homologous to the corolla which is on the verge of extinction.

Embryological studies in the family are meagre. Development of the gametophytes has been studied by Ballantine (1909) in *Protea lepidocarpon*, Messeri (1928), Brough (1933), Kausik (1938a) in species of *Grevillea*, Kausik (1940) in *Hakea saligna* and by Jordaan (1916) and Garside (1946) in *Brabeium stellatifolium*.

Berry (1916) from fossil evidence, Lancaster (1952) from cytological studies and Levynns (1958) from a study of the phytogeographic distribution of the African Proteaceae, favour a northern origin of the family and its southward migration.

Due to the meagre cytological, floral anatomical and embryological studies, the Proteaceae remains one of the incompletely understood families of angiosperms.

The following points require clarification :

- (a) *Morphological* : Are the flowers in Proteaceae primitively monochlamydeous or simple due to reduction? What is the nature of the perianth and the morphology of the nectary? What is the significance of the arrangement of the flowers regularly in pairs in Grevilloideae?
- (b) *Taxonomic* : How far are the tribes as at present constituted natural and homogeneous? Do all genera known to date fit into the existing tribes? What are the evolutionary tendencies among the tribes and within the family?
- (c) *Phylogenetic* : Is the family monophyletic or polyphyletic? Monotopous or polytopous? What are its affinities and evolutionary potentialities?
- (d) *Distributional* : How is the present disjunct distribution of the family to be accounted for? Does the present day geographic distribution or geological history throw any light on the time and place of origin of the family?

In a previous article, the writer (Venkata Rao, C. 1957) postulated the criteria of taxonomic value made use of in the revision of the family by him, and also

TABLE I
Materials studied

No.	Genus	Total no. of species	Geographic distribn.	Species examined	Source
1.	<i>Bellendena</i> Br.	1	Tasmania	<i>B. montana</i> Br.	! Tasmania
2.	<i>Persoonia</i> Sm.	72	c.43 East Austr. c.27 West Austr. 2 Tasmania 1 New Zealand	<i>P. virgata</i> Br. <i>P. nutans</i> Br. <i>P. salicina</i> Pers. <i>P. ferruginea</i> Sm. <i>P. myrtilloides</i> Sieb. <i>P. oxycoccoides</i> Sieb. <i>P. lanceolata</i> Andr. <i>P. pinifolia</i> Br. <i>P. linearis</i> Andr. <i>P. chamaepeuce</i> Lhotk. <i>P. rigida</i> Br. <i>P. caleyi</i> Br. <i>P. microcarpa</i> Br. <i>P. longifolia</i> Br. <i>P. saundersiana</i> Kipp. <i>P. succata</i> Br. <i>P. juniperina</i> Labill. <i>P. gunnii</i> Hook.f. <i>P. toru</i> Br.	F.H. Mr. L. S. Smith, Queensland N.S.W., R.H. do do do N.S.W. N.S.W. N.S.W. and Vic. R. H. N.S.W. R.H. do N.S.W. and Vic. R. H. West Aus. R.H. do do !do !Tas. !Tas. H. New Zealand
3.	<i>Cenarrhenes</i> Labill.	2	1 Tasmania 1 New Caledonia	<i>C. nitida</i> Labill.	!Tasmania
4.	<i>Agastachys</i> Br.	1	Tasmania	<i>A. odorata</i> Br.	!Tasmania
5.	<i>Symphyonema</i> Br.	2	N.S.W.	<i>S. paludosum</i> Br. <i>S. montana</i> Br.	R.H. H. Melbourne Herbarium
6.	<i>Beauprea</i> Brong. et Gris.	c.8	New Caledonia	<i>B. paniculata</i> <i>B. pancheri</i>	F.M. Inspector of Forests, Noumea, New Caledonia.
7.	<i>Garnieria</i> Brongn. et. Gris.	1	New Caledonia	Nil	
8.	<i>Dilobeia</i> Thou.	1	Madagascar	Nil	
9.	<i>Brabeium</i> L.	1	S. Africa	Nil	

Abbreviations used : !collected by the writer; R.H.=Rodway Herbarium; F.M.=fixed material; H=herbarium specimen; N.S.W.=New South Wales, Vic.=Victoria.

gave a synopsis of the new classification. In the present studies he proposes to describe his observations on cytology, morphology, floral anatomy and embryology of a large number of members examined and discuss the various problems posed above.

MATERIALS AND METHODS

The present studies were made on fresh material collected by the writer during his stay and tour of Tasmania and Australia and also materials obtained from other sources which are acknowledged below. Where fresh material was not available, herbarium material was used for morphological and floral anatomical studies. The genera, number of species in each genus, their geographic distribution, the species examined and their source are given in Table 1.

For cytological studies, aceto-iacmoid smears of flower primordia, root tips and young anthers were examined. Microtome sections stained in Crystal Violet according to the schedule given by Darlington and LaCour (1955) were also used. For floral anatomical and embryological studies, materials fixed in formalin acetic alcohol or even in 70 per cent alcohol were used with satisfactory results. Delafield's Haematoxylin, Safranin and Fast Green or Crystal Violet and Erythrocin were used as stains. For a study of endosperm haustoria whole mounts were made according to the method given by Kausik (1938, 1942). Studies in anatomy of vegetative parts were also made in a few cases; both microtome and free hand sections, stained in Safranin and Fast Green were used.

Where herbarium material was used, it was soaked overnight in 5 per cent teepol solution for morphological investigations. For microtoming, the material was kept in 1 per cent solution of caustic soda at 50°C for 12-24 hours, washed thoroughly and then treated like freshly fixed material.

CYTOLOGY

Though Bentham (1870) described the stamens in *Bellendena montana* as 'all perfect', the writer noticed that it shows a gynodioecious system of sex distribution i.e., the occurrence of male sterile (functionally female) and hermaphrodite flowers on separate plants. Unlike in the gynodioecious species of New Zealand *Fuchsia* (Godley, 1955), the flowers in *Bellendena* are morphologically indistinguishable. In Proteaceae, partial male sterility (i.e., sterility of one or more stamens of a flower) is noticed in *Persoonia hakeaeformis*, *Adenanthos* sp., *Synaphea*, *Conospermum*, *Placospermum* and *Protea* sp. and dioecy in *Leucadendron* and *Heliciopsis*, but *Bellendena* is so far the only member in which gynodioecy is reported.

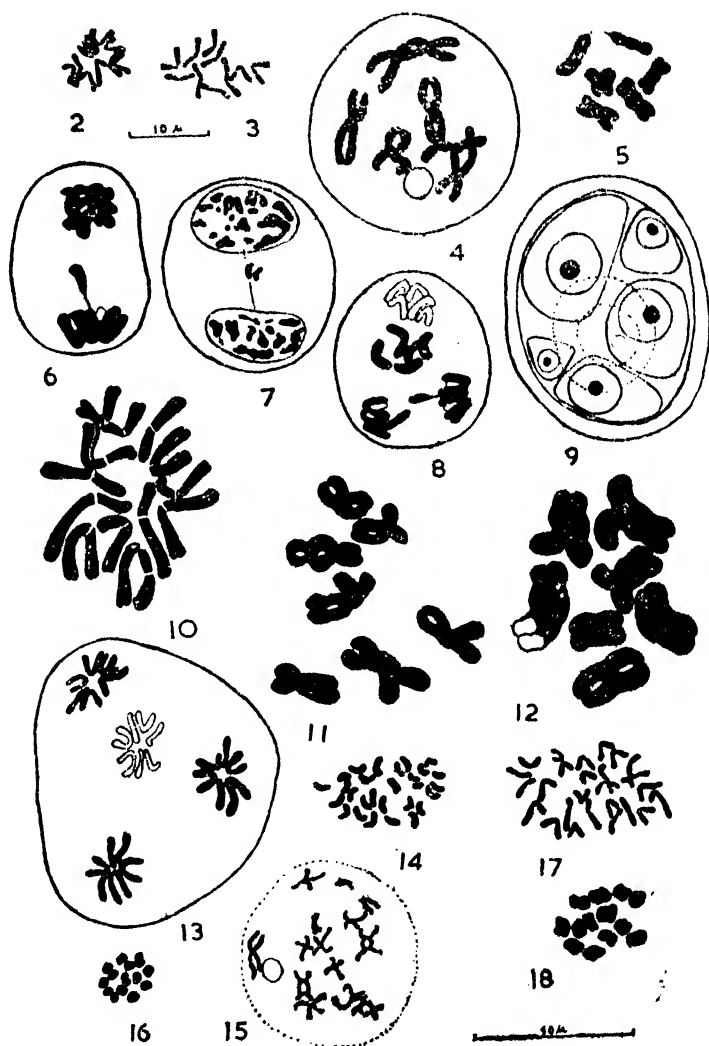
A study of populations of *Bellendena* growing on Mt. Wellington and Mt. Field National Park showed female percentages ranging from 34.5 to 48.0 as shown in Table 2.

TABLE II
Gynodioecy in Bellendena montana

No.	Locality	Male sterile plants		Total population studied
		Number	Percentage	
1.	Mt. Wellington/Summit	45	45.0	100
2.	do First Marsh	40	34.5	115
3.	Mt. Field National Park-Dobson Huts	47	37.7	135
4.	do Wombat moor	59	48.0	123

Gynodioecy is a stable genetic system which promotes outbreeding since the female flowers must necessarily be fertilised by the hermaphrodites. It is reported in several unrelated families of angiosperms viz., Acanthaceae (*Sautiera hortensis*)

Compositae (*Cirsium oleraceum*), Labiateae (*Origanum vulgare*) and Gramineae (*Lolium perenne*). Allen (1940) believed that gynodioecy is derived from hermaphroditism and is transitional to dioecy, but Lewis (1942) considered it to be an independent sexual system and not a step towards dioecism because in Labiateae which contains about 90 per cent of the reported gynodioecious species, there are



FIGS. 2-18.—Figs. 2-9. *Bellendena montana*. Fig. 2. Mitotic metaphase plate from section of root tip, c.v. stain. Fig. 3 Mitotic metaphase plate from smear of root tip after cold treatment; note banded appearance of the chromosomes. Fig. 4. Late prophase from p.m.c. Fig. 5. Metaphase I showing terminal association and precocious separation of homologues of a pair. Fig. 6. Late anaphase I showing formation of a fragment. Fig. 7. Telophase I showing division of fragment into chromatids. Fig. 8. Anaphase II showing formation of a fragment. Fig. 9. A polyad of microspores. Figs. 10 and 11. *Persoonia juniperina*. Fig. 10. A mitotic metaphase plate from tapetal cell. Fig. 11. Metaphase I from p.m.c. Figs. 12 and 13. *P. gunnii*. Fig. 12. Metaphase I from p.m.c. Fig. 13. Late anaphase II. Figs. 14-16. *Cenarrhenes nitida*. Fig. 14. Mitotic metaphase plate from a tapetal cell. Figs. 15 and 16. Prophase I and Metaphase II from p.m.c. Fig. 17 and 18. *Agastachys odorata*. Fig. 17. Mitotic metaphase plate from cell of flower primordium. Fig. 18. Metaphase I from p.m.c.

no dioecious species at all. From the classical experiments of Correns (1928) which showed that both the females and hermaphrodites breed true, it was believed that gynodioecism is under the control of a permanent cytoplasmic particle or plasmagene. However, from a study of the breeding system in *Origanum vulgare*, Lewis and Crowe (1956) conclude that gynodioecism in this species is controlled by two independent genes.

The chromosome numbers in *Bellendena montana* as determined from squashes of young anthers and root tips are $n=5$ and $2n=10$. The chromosomes range from $4-6\mu$ in length and are relatively thick (Fig. 2). One pair is distinctly longer than the rest and shows a sub-median constriction; one pair is short and the rest intermediate in size. No secondary constrictions or trabants are noticed. Such simple karyotypes are usually seen in primitive plants. Chromosomes of the root tips after 24 hours of cold treatment at 0°C showed a banded appearance suggesting the presence of heterochromatic segments (Fig. 3) as Smith-White (1955) also found in *Leucopogon juniperinus*.

In the majority of the p.m.c. pairing and disjunction of chromosomes are normal and 1-4 chiasmata are noticed in each bivalent (Fig. 4). The X-ta frequency obtained from a count in 10 cells chosen at random is 2.1 per pair. In less than 1 per cent of the cells, one pair of chromosomes showed irregular pairing which ranged from a slight terminal association and precocious separation (Fig. 5) to the absence of pairing. In a few cases a chromosome bridge and a fragment are noticed during anaphase I or II (Figs 6 and 8), which show that one of the pairs is heterozygous for an inversion. The fragment forms micronuclei and super-numerary microspores after or without division into chromatids so that polyads with 5-7 microspores were noticed (Figs. 7 and 9). The spores formed by the micronuclei and those formed by nuclei deficient in the full chromosome complement give rise to sterile pollen grains. These form less than 10 per cent of the grains of fertile anther loculi. Lawson (1930) reported the occurrence of larger percentages of sterile pollen in several mainland species of the Proteaceae.

The chromosome numbers in *Persoonia gunnii* and *P. juniperina* as determined from squashes of young anthers, are $n=7$ and $2n=14$ (tapetal cells). The chromosomes are relatively thicker and longer than those of *Bellendena* and showed no secondary constrictions or trabants (Fig. 10). Pairing and disjunction of chromosomes are normal (Figs. 11-13). Lancaster (1952) reported the same numbers in some mainland species of *Persoonia*. She also found that a secondary constriction occurs in one of the pairs near to the centromere.

Smears of the p.m.c. of *Cenarrhene nitida* showed 14 bivalents and tapetal cells showed $2n=28$ (Figs. 14-16). The chromosomes are short and somewhat thick. Meiotic divisions proceed normally.

In *Agastachys odorata* the chromosome numbers are $n=13$ and $2n=26$ (Figs. 17 and 18). The meiotic divisions proceed normally. The chromosomes are thinner than those of *Bellendena* and more elongated than those of *Cenarrhene*.

INFLORESCENCE AND FLOWER

In several species of *Persoonia* (e.g., *P. saccata* and *P. juniperina*), the flowers are solitary axillary and diffusely scattered (Plate XVIII, 8, 9). In *P. pinifolia* they are aggregated into terminal racemes in which region the leaves are small and bract like (Plate XVIII, 10). In *Symphyonema* and *Beauprea* the inflorescence is a lax panicle (Plate XVII, 6; Figs. 36 and 42), while the spikes are more closely clustered in *Cenarrhene* and *Agastachys* (Plate XVII, 3, 4, Plate XVIII 7). It is a simple raceme in *Bellendena* (Plate XVII, 1, 2).

The flowers are ebracteate only in *Bellendena*. The young inflorescence is covered over and protected by a group of bud scales (modified leaf bases) which

are left behind as the inflorescence elongates (Figs. 19-22). In other genera there are relatively large and persistent bracts (Figs. 37, 39, 44 and 46).

The flowers in all genera are actinomorphic (cf. Fig. 26). They are zygomorphic only in *Picnostylis* section of *Persoonia* (e.g., *P. saundersiana* and *P. saccata*) due to the presence of saccate perianth and curved style (Figs. 30-33). In *Bellendena*, *Persoonia* and *Agastachys* the flowers are glandless. In other genera there is a hypogynous nectary with 4 alternitepalous, vascularised lobes (Figs. 27, 29, 38, 40, 45 and 47). The stamens are free from the tepals only in *Bellendena* (Figs. 23) and 54). In all other genera they are adnate to the bases of tepals (Figs. 25, 31). In *Symphyonema* the filaments are connate at the top (Fig. 44). In *Agastachys*, *Symphyonema* and *Amblyanthera* section of *Persoonia*, the stamens are non-apiculate (Figs. 25 and 28). In other members they have produced connectives (Figs. 31, 32, 34). In *Cenarrhenes nitida* and *Beauprea paniculata*, the connective of the posterior stamen is elongated and tapering (Figs. 46, 110 and 122). In *Bellendena* and *Persoonia* the ovary is stipitate. It is sessile or nearly so in other members. There are two orthotropous pendulous ovules in *Bellendena* (Fig. 24) *Persoonia* and *Symphyonema*. In other genera there is a single ovule per carpel, which is pendulous in *Cenarrhenes* and *Agastachys* and lateral in *Beauprea* (Fig. 38). The style is terminal and stigma simple (Figs. 27 and 43), or hook like (Fig. 35). In *Agastachys* it is 2-lobed (Fig. 41). The fruit is a 1-seeded achene in *Bellendena*, a succulent drupe in *Persoonia* and *Cenarrhenes* (Plate XVII, 5; II, 9, 10) and a samara in *Agastachys* (Figs. 270, 271).

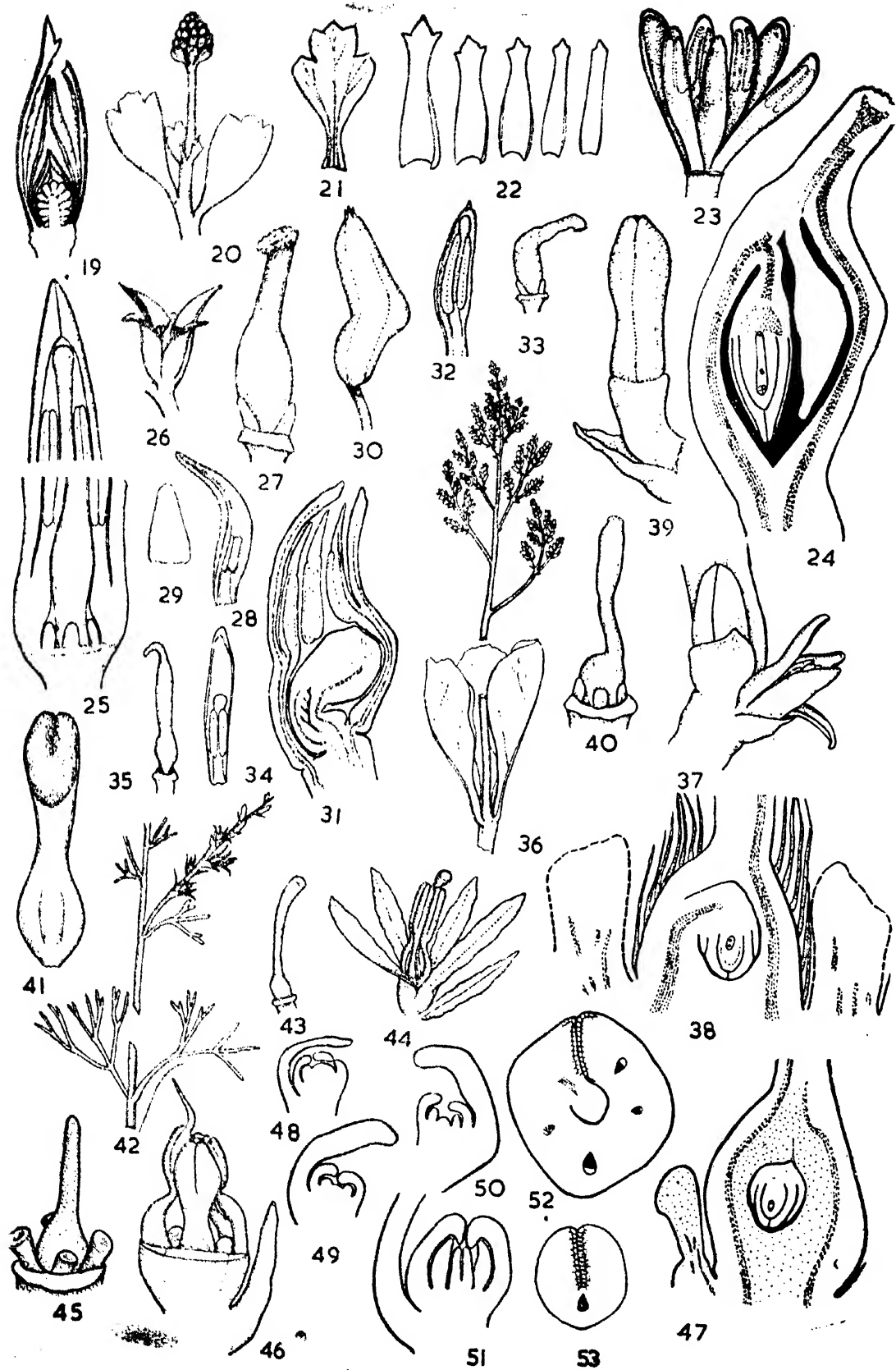
ORGANOGENY

The floral organs arise in acropetal succession (Figs. 48-51). The margins of the carpel fuse incompletely in the young ovary (Figs. 52, 53, 156 and 157). The line of fusion remains distinct for some time during the growth of the ovary (Figs. 62 and 72).

FLORAL ANATOMY

In *Agastachys* the stele of the peduncle is 3-angled. Since the vascular supply for the flowers is abstricted from each of the ridges (Fig. 100), the flowers stand in three vertical rows. The bract is 1-traced in *Persoonia* and *Beauprea* (Figs. 80, 111 and 117), and 3-traced in *Symphyonema*, *Cenarrhenes* and *Agastachys*. The traces cause separate gaps in the floral stele (Figs. 90-93). In *Cenarrhenes*, unlike in *Agastachys* the bract laterals depart earlier than the bract midrib (Figs. 101, 103, 123-125).

The pedicel shows either 4 vascular bundles as in *Bellendena* and *Beauprea pancheri* (Figs. 55 and 117) or a ring of more than 4 bundles or a siphonostele as in *Persoonia* sp. which breaks up into 4 arcs (Figs. 67 and 80). In *Symphyonema* there are only 3 vascular bundles at the base of the flower which increase in number by splitting in the thalamus region (Figs. 88-90). The flowers in *Bellendena* present the simplest structure anatomically. The 4 bundles of the pedicel bend outwards in the thalamus; strands are given off from the margins of the lateral and anterior bundles (Figs. 56 and 57). These bend inwards, fuse suitably and reorientate as 3 carpellary traces (Fig. 58). There is no branching of the bundles in the ovary wall or pericarp. The 4 peripheral bundles divide tangentially into 2 each. The outer bundles thus formed function as the tepal traces; each expands tangentially and divides into 3 bundles, which traverse as the midrib and marginals (Figs. 58-60). The inner ones function as staminal traces; these are concentric for most part and quite free from the tepal bundles from the base (Figs. 60 and 61). The ventral



carpellary bundles give off ovular traces (Figs. 62) and fade out towards the base of the style. The dorsal bundle extends to the top of the style and becomes associated with patches of sclerenchyma (Figs. 63 and 64). The core of the style is filled with glandular transmitting tissue (Fig. 65).

In other members studied, though the floral structure is essentially similar, some variations are noticed. The tepals are antero-posterior in all *Persoonieae* studied. In *Persoonia* and *Beauprea pancheri*, the tepals are 3-bundled as in *Bellendena* (Figs. 68-70, 75-77, 82-85, 118-121). In others the tepal trace traverses the tepal without branching (Figs. 93-96, 98, 102-104, 109, 115, 126-128). The staminal trace is usually organised as a pair of bundles to the inside of the tepal trace (Figs. 68, 74, 75, 78, 79, 83 and 112). The twin bundles of each trace fuse together either before or after emergence into the tepal (Figs. 69, 82, 93, 103 and 113). In any case they do not unite with the tepal trace so that the tepal and stamen show only congenital concrescence but not true adnation (Figs. 66, 87, 99, 110 and 122). The stamens in all members show distinct filaments (Figs. 106, 107, 114, 120, 121 and 128). The filaments in *Symphyonema* are flat and become connate by marginal hairs (Figs. 96 and 97) as the tepals do in all *Proteaceae*. In *Persoonia saccata* (Fig. 86), *Cenarrhenes* and *Beauprea* sp. the staminal bundle extends into the produced connective.

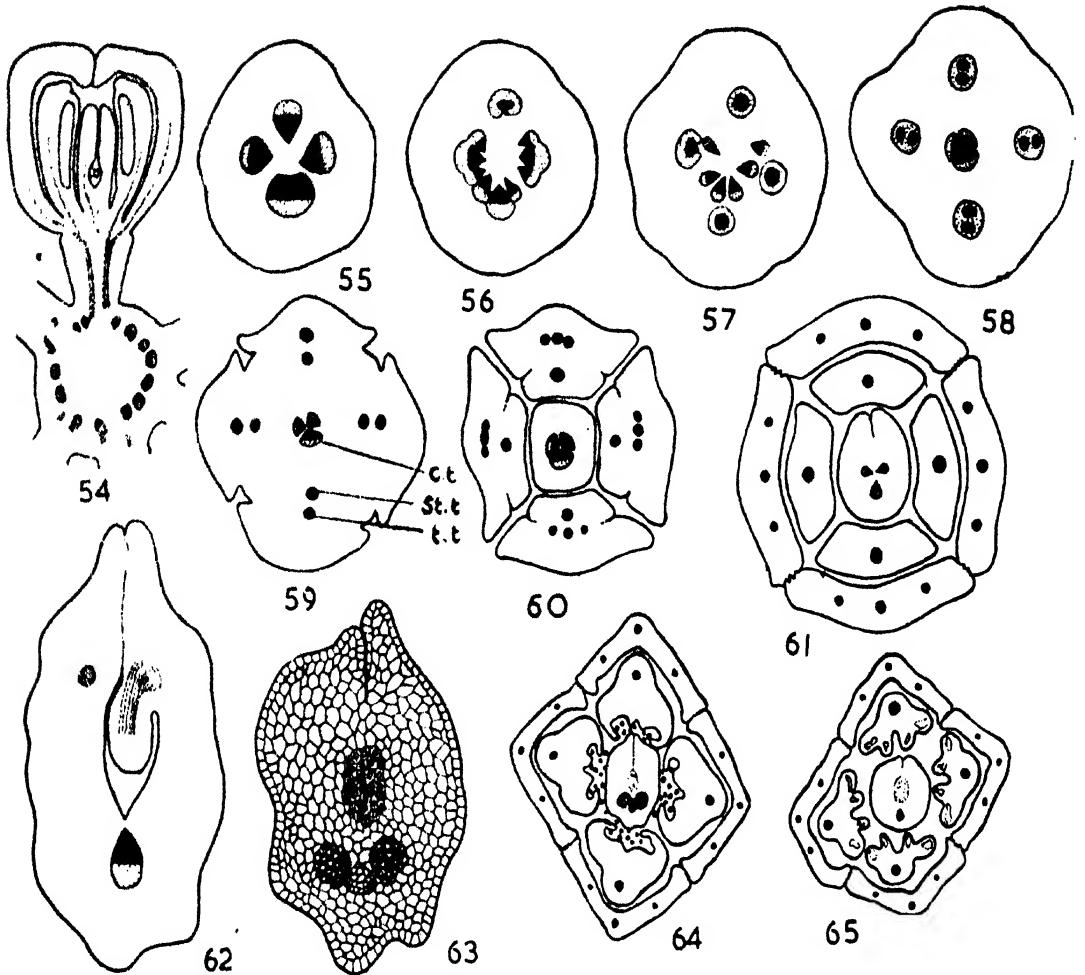
In *P. saccata* usually the two bundles of each staminal trace fuse together just below the level of separation of the filament from the tepal. In one abnormal flower, however, the bundles of the lateral traces not only remained separate but entered the filaments of independent stamens so that the flower showed six stamens and not the customary four (Fig. 83-85).

In *Persoonia* sp. the lobes of the nectary are vascularised by strands derived either from the main stele or from the tepal laterals or from staminal trace (Figs. 69, 74-76 and 81). In *Cenarrhenes* and *Beauprea* they are derived from the outer margins of the vascular bundles in the alternitepalous sectors of floral stele (Figs. 112, 113, 118, 119, 125 and 126). As these bundles branch further in the base of the gland, 4-7 strands are noticed in each lobe (Figs. 70, 71, 76, 82, 116 and 127). The vascular bundles are surrounded by richly protoplasmic and tannin bearing cells (Fig. 71).

In *Beauprea paniculata* the carpel is 3-traced as in *Bellendena* (Fig. 114). In *Persoonia* and *Symphyonema* it is 5-traced while in *Cenarrhenes* it is 7-traced and there may be further branching in the ovary wall. The dorsal bundles and sometimes the median dorsals also extend into the style (Figs. 73, 97, 106, and 115).

EXPLANATION OF FIGURES

Figs. 19-53.—Figs. 19-46. Floral structure in *Persoonieae*. Figs. 19-24. *Bellendena montana*. Fig. 19. L. S. young inflorescence and surrounding bud scales, $\times 8$. Fig. 20. A twig with inflorescence, $\times 8$. Fig. 21. A cleared leaf showing venation, $\times 1$. Fig. 22. Bud scales, $\times 8$. Fig. 23. Flower with two tepals removed, $\times 4$. Fig. 24. L.S. mature ovary, $\times 40$. Figs. 25-35. *Persoonia* sp. Fig. 25. L. S. flower bud of *P. lanceolata*, $\times 8$. Figs. 26-29. *P. toru*. Fig. 26. An open flower, $\times 2$. Fig. 27. Pistil and nectary, $\times 8$. Fig. 28. A tepal and the attached stamen, $\times 4$. Fig. 29. A lobe of the nectary, $\times 8$. Figs. 30-33. *P. saccata*. Fig. 30. A flower bud, $\times 2$. Fig. 31. L. S. flower bud, $\times 4$. Fig. 32. A tepal and attached stamen, $\times 2$. Fig. 33. Pistil and nectary, $\times 2$. Figs. 34 and 35. *P. microcarpa*. Fig. 34. A tepal and attached stamen, $\times 3$. Fig. 35. Pistil, $\times 3$. Figs. 36-40. *Beauprea* sp. Fig. 36. A branch with inflorescence of *B. paniculata* $\times 4$. Fig. 37. A flower, $\times 4$. Fig. 38. L. S. ovary and nectary $\times 60$. Figs. 39 and 40. *B. pancheri*. Fig. 39. A flower bud, $\times 4$. Fig. 40. Ovary and nectary, $\times 4$. Fig. 41. Pistil of *Agastachys*, $\times 25$. Figs. 42-44. *Symphyonema faludosum*. Fig. 42. A branch with inflorescence, $\times 1$. Figs. 43 and 44. Pistil and flower, $\times 10$. Figs. 45-53. *Cenarrhenes nitida*. Fig. 45. Pistil and nectary, $\times 6$. Fig. 46. Flower after removing tepals, $\times 6$. Fig. 47. L. S. ovary and one lobe of nectary, $\times 15$. Figs. 48-51. Stages in the organogeny of flower, $\times 30$. Figs. 52 and 53. T. S. ovary and style, $\times 25$.



FIGS. 54-65.—*Bellendena montana*. Fig. 54, L. S. flower. Fig. 55, T. S. pedicel, Figs. 56-61, Transverse sections through flower bud at various heights. Fig. 62, T. S. carpel. Fig. 63, T. S. style. Figs. 64 and 65, T. S. through top of hermaphrodite and male sterile flower buds. *tt.*=tepal trace; *st.t.*=staminal trace; *ct.*=carpellary traces. Fig. 54, $\times 25$; Figs. 55-61 and 63, $\times 40$; Fig. 62, $\times 65$; Figs. 64 and 65, $\times 12$. (Explanation in text).

Although in species of *Persoonia* the carpel is usually two ovulate, in an abnormal ovary of *P. saccata*, three ovules were seen (Figs. 31 and 221). This resembles the carpel of *Garnieria* (New Caledonia), the only genus of Persoonieae to show the multiovulate condition. In the 2-ovulate carpels, the ovular traces are derived alternately from both ventrals. In *Cennarrhens* and *Beauprea*, though the second ovule is suppressed, the marginal bundle that fed it still persists. In the former a parenchymatous protuberance (vestigial ovule) is noticed on the sterile carpellary margin (Figs. 239 and 241), and occasionally a normal second ovule is developed (Figs. 258). This shows that the uniovulate condition is derived by suppression of the extra ovules. The vasculature of the carpel in *Agastachys* is interesting in this connection. It is atypical in showing 4 traces which traverse as the dorsal, 2 median laterals and one ventral bundle, there being no bundle in the sterile carpellary margin (Figs. 103 and 104). From this it is evident that in *Agastachys* reduction has gone further; the second ovule as well as the marginal that had been

feeding it are completely suppressed. The surviving marginal directly functions as the ovular trace (Fig. 105). The dorsal bundle fades at the base of the style and each of the median laterals extends into one lobe of the stigma (Figs. 106-108). There is no branching of the ovarian bundles even in the fruit wall (Fig. 271).

In *Cenarrhenes*, though normally the ventral suture points to the posterior side (Fig. 128), sometimes a tendency is noticed for the torsion of the ovary (Fig. 129).

MICROSPOROGENESIS AND MALE GAMETOPHYTE

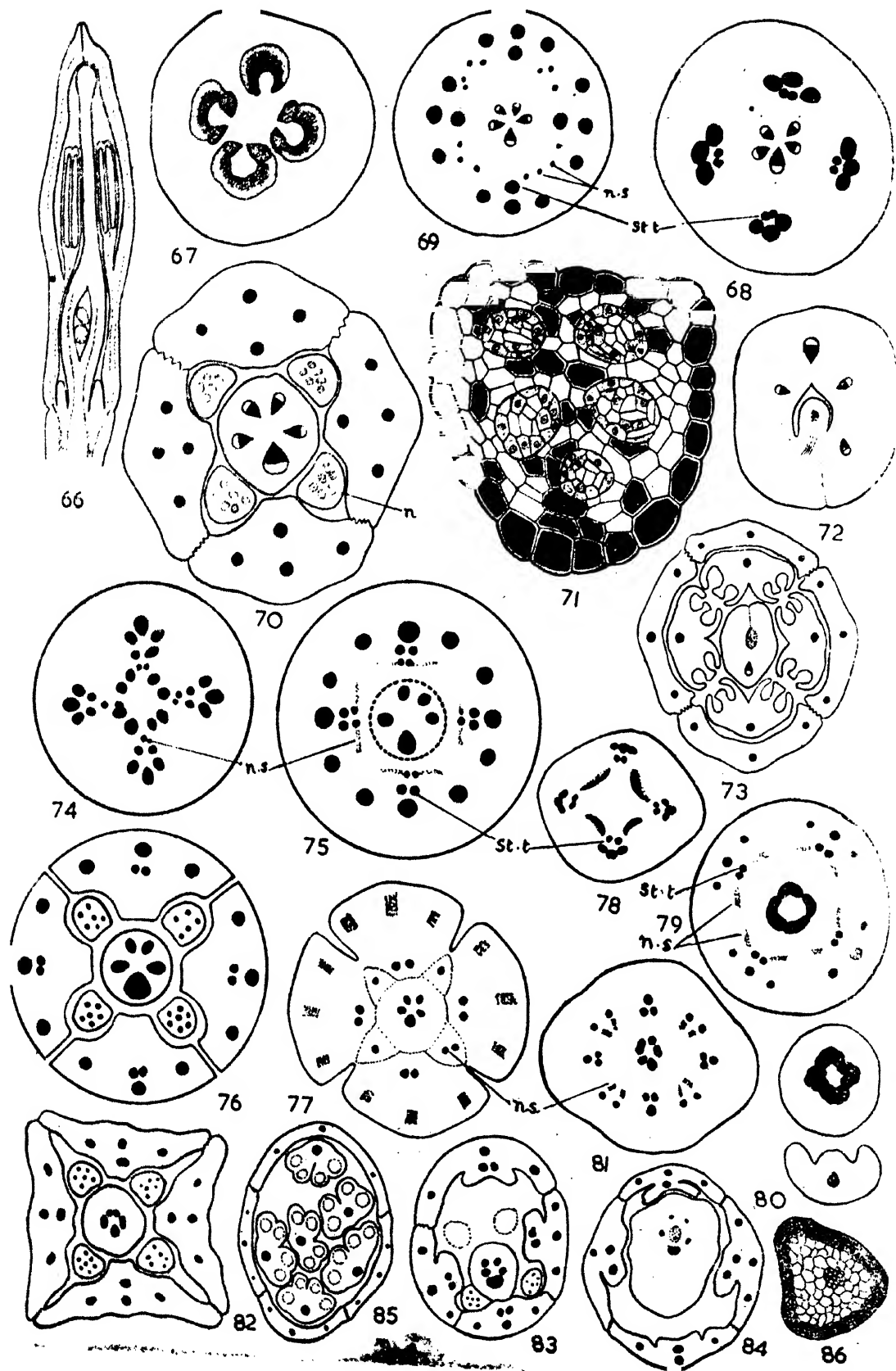
In all species studied, the anthers are 4-locular. The primary archesporium differentiates as 1 or 2 rows of hypodermal cells in each lobe. These divide periclinally forming the primary parietal cells to the outside and the primary sporogenous cells to the inside. The former give rise to 4-5 layers of wall cells below the epidermis (Figs. 130, 131 and 147). The sub-epidermal layer develops into the fibrous endothecium and the innermost into the tapetum of the secretory type (Figs. 133, 151 and 154). The septum between the anther loculi persists for some time after the dehiscence of the anther; in *Agastachys* there is no distinct septum (Figs. 154).

There is a secondary increase of the sporogenous tissue of the anther (Figs. 130, 131 and 147). Microspore tetrads are usually tetrahedral. Cytokinesis is of the simultaneous type and is brought about by furrowing. In all genera studied, the pollen grains are triporate and triangular except in *P. saccata* (Fig. 143) in which they are nearly spherical. The exine is smooth in *Beauprea* and *Bellendena* (Figs. 146 and 152). In others it ranges from finely granular to markedly reticulate pattern (Figs. 135, 136, 138 and 153). The intine may be uniformly thick as in *P. juniperina*, *P. saundersiana* and *P. lanceolata* (Figs. 134, 136 and 138) or thicker in the region of the germ pores and slightly or markedly protruding through them as in *Cenarrhenes*, *P. salicina*, *P. linearis*, and *P. myrtilloides* (Figs. 132, 135, 140 and 141). In *P. virgata*, *P. ferruginea*, *P. oxycoccoides* and *P. microcarpa* the intine protrudes through the germ pores and forms knob like swellings (Figs. 137, 142, 144 and 145). Pollen grains which are hemi-spherical on one side are noticed in *P. lanceolata* (Fig. 139). The pollen is 2-celled at the shedding stage. The generative cytoplasm is devoid of starch and is seen as a hyaline sheath around the generative nucleus in the earlier stages (Fig. 155). Later, the generative nucleus becomes ellipsoidal (Fig. 134 and 137) and the generative cytoplasm becomes obscure.

In pollen sterile anthers of *Bellendena* the microsporocytes degenerate without undergoing meiotic divisions. During early prophase I they separate out from each other, become covered with a thick wall and begin to show signs of degeneration. The cytoplasm of the tapetal cells also disintegrates and the cells become filled with droplets of deep staining material (Figs. 148 and 149). The hypodermal cells of the anther wall do not develop fibrous thickenings and the anthers do not dehisce (Fig. 150).

OVULE

The ovule primordia arise transverse to the ovarian loculus and become pendulous with growth (Figs. 156, 158-160, 168, 223 and 228). The funicles of the ovules in *Bellendena* and *Persoonia* (Fig. 222) are relatively longer than those of *Cenarrhenes* and *Agastachys* (Figs. 245, 246 and 248), while the funicle of the laterally attached ovule of *Beauprea* is much shorter (Fig. 252). In *Persoonia*, the funicle of one ovule is much longer than that of the other and this difference facilitates the accommodation of the ovules in the space of the loculus (Fig. 223). The vascular bundle of the funicle branches in the chalaza and forms a ring of bundles (Figs. 199,



211 and 233). The ovules are orthotropous, bitegmatic and crassinucellate (Figs. 218-220). The inner integument arises earlier than the outer (Figs. 161-163, 237-239). In the mature ovules, the outer integument remains biseriate while the inner becomes about 4 cells thick. The inner alone forms the micropyle which is usually long and straight (Figs. 165, 168, 208, 211 and 249). In *Persoonia* sp. the micropyles press against the side or base of the loculus and become bent or asymmetrical (Fig. 222). In the multiovulate carpel of *P. saccata* the integuments and micropyle are not normally formed due to exigencies of space (Fig. 221). The primary parietal cell of the ovule gives rise to 4-5 layers of parietal tissue; the cells of nucellar epidermis may also divide periclinally and form a nucellar cap (Figs. 165, 224, 240, 247 and 252). Three or more layers of these cells persist in the mature ovules and developing seeds (Figs. 169, 212 and 250). In *Bellendena* they not only persist till a late stage in seed development but become glandular (Figs. 184-186). In *Bellendena* and *Persoonia* sp. the cells of nucellus around the antipodal end of the embryo sac become thick walled and form a postament in the developing seeds (Figs. 174, 176, 224, 230-233). A transverse section of the postament of *Bellendena* shows some cells radiating from it (Fig. 177). In *Persoonia* sp., *Agastachys* and *Beauveria*, the antipodal end of the embryo sac extends to the chalaza. In *Cenarrhones* and *P. lanceolata*, it is separated from the chalaza by several layers of nucellar cells. A hypostase of elongated regularly placed cells extends between the antipodal end of the sac and the chalaza. The cells are thick walled in *Persoonia* species (Figs. 230-232) but thin walled and glandular in *Cenarrhones nitida* (Figs. 245 and 256). Tannin is accumulated in the cells of the chalaza and outer epidermis of the outer integument and inner epidermis of the inner integument.

MEGASPOROGENESIS AND FEMALE GAMETOPHYTE

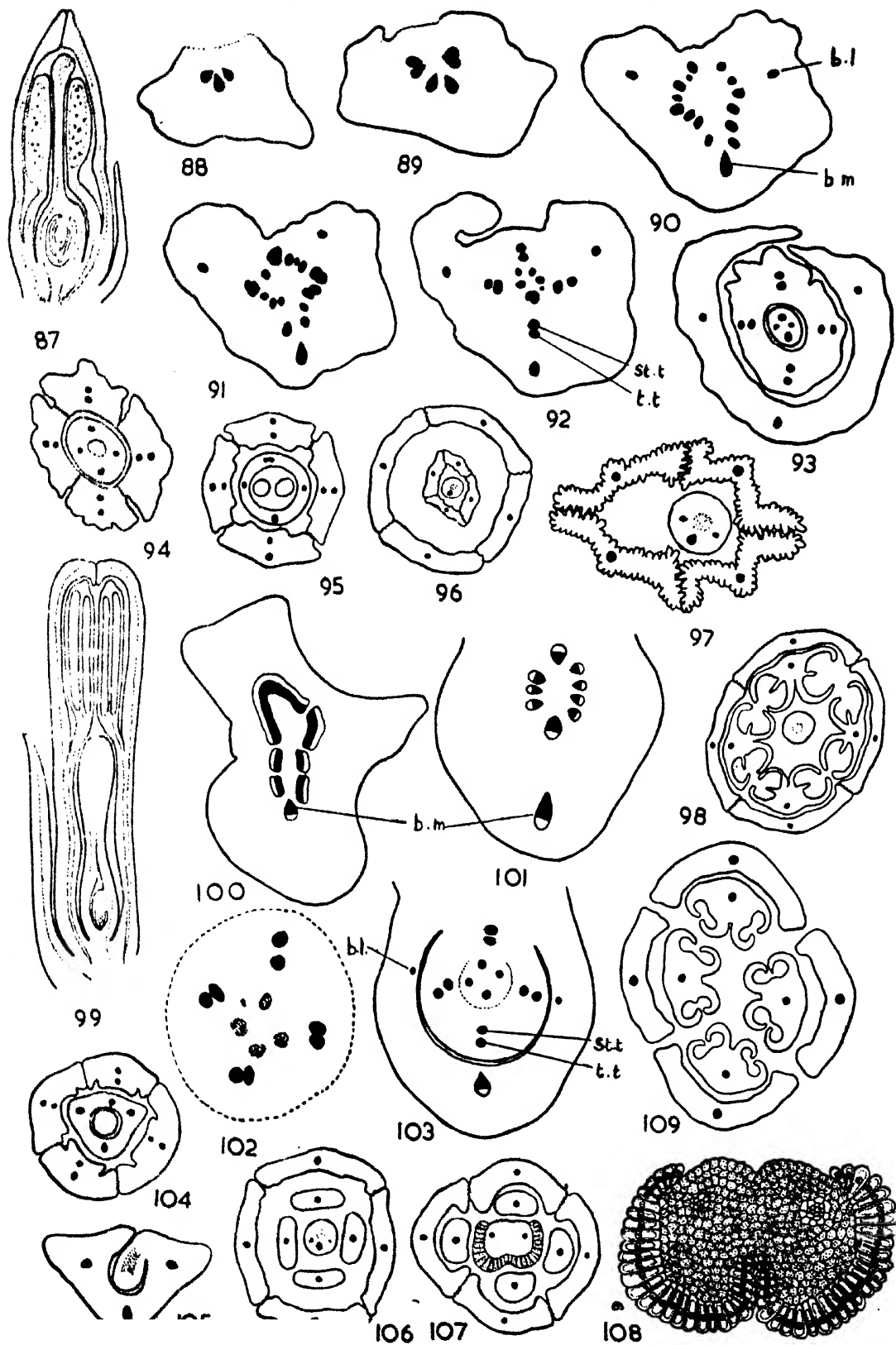
The archesporium of the ovule consists of the single hypodermal cell (Figs., 161 and 227). This cuts off the primary parietal cell (Fig. 251) and functions as the megaspore mother cell which becomes elongated and tapering when full grown (Figs. 162, 163, 207, 229 and 239). A linear tetrad of megaspores is formed (Figs. 164, 208, 241-244); the lowest megaspore functions and forms the embryo sac according to *Polygonum*-type (Figs. 165, 166, 209, 210 and 232). The synergids show filiform apparatus and rounded or hook like protuberances on their free sides (Fig. 167). The polar nuclei do not fuse before fertilization (Fig. 170). The antipodals are inconspicuous but persist till a few endosperm nuclei are formed (Fig. 173). The cytoplasm of the embryo sac shows starch.

FERTILIZATION

Bellendena, *Cenarrhones* and *Agastachys* are very showy when in flower. The flowers of *Bellendena* give out a foetid smell which is attractive to some insects. The flowers are protandrous and the pollen is shed on the stigma long before the

EXPLANATION OF FIGURES

Figs. 66-86. *Persoonia* sp. Figs. 66-73. *P. juniperina*. Fig. 66. L. S. flower bud. Figs. 67-70 and 73. sections at various heights through flower bud. Fig. 71. T. S. a lobe of the nectary, Fig. 72. T. S. carpel. Figs. 74-76. Semidiagrammatic transverse sections of flower bud of *P. lanceolata*. Fig. 77. T. S. flower bud of *P. virgata*. Figs. 78 and 79. *P. gunnii* Figs. 80-82. *P. pinifolia*. Figs. 83-86. *P. saccata*. Figs. 83-85. Sections through an abnormal flower with six stamens. Fig. 86. T. S. appendage of stamen. *n.s.*=nectary strands; *st.t.*=staminal trace; *n*=lobe of nectary. Fig. 66, $\times 8$; Figs. 67-70, 72 and 77, $\times 40$; Fig. 71, $\times 160$; Fig. 73, $\times 35$ Figs. 78 and 79, $\times 28$; Figs. 80-82, $\times 20$; Figs. 83-85, $\times 6$; Fig. 86, $\times 85$. (Explanation in text).



flowers open. Self sterility prevents self fertilization. Pollen grains are caught among the glandular stigmatic hairs (Fig. 225) where they germinate in a monosiphonous manner (Figs. 171 and 172). The transmitting tissue of the style facilitates the passage of pollen tubes. In several species of *Persoonia* the transmitting tissue is biseriate (Fig. 206) and the cells adjoin the glandular tissue lining the loculus. In *P. saccata* the transmitting tissue is more extensive (Fig. 226). In *Bellendena* the ovules hang freely in the loculus so that the pollen tubes have to bridge a gap in order to reach the micropyles (Fig. 24). In *Beauprea* and *Agastachys* the micropyles stand close to or in contact with the base of the loculus. In *Persoonia* sp. the glandular cells of the loculus adjacent to the micropyle proliferate and form some glandular tissue which functions as an obturator (Figs. 205, 212, 230 and 231). In *Cenarrhenes*, the epidermal cells of the inner integument around the micropyle become papillate and come in contact with similar cells lining the base of the loculus. Together they seem to facilitate the passage of pollen tubes (Fig. 245). After fertilization the micropyle in *Cenarrhenes* becomes conical and fits into the funnel shaped base of the loculus; the surrounding cells become glandular and seem to assist in the nutrition of the embryo sac (Fig. 255, 257 and 260).

The pollen tube enters the ovule porogamously and usually a synergid becomes effected when it enters the embryo sac (Figs. 170 and 254); in *Cenarrhenes*, remnants of such synergids are seen by the side of growing embryos. In *Bellendena* the pollen tubes are narrow and ephemeral; in *Cenarrhenes* they are relatively wider and more persistent.

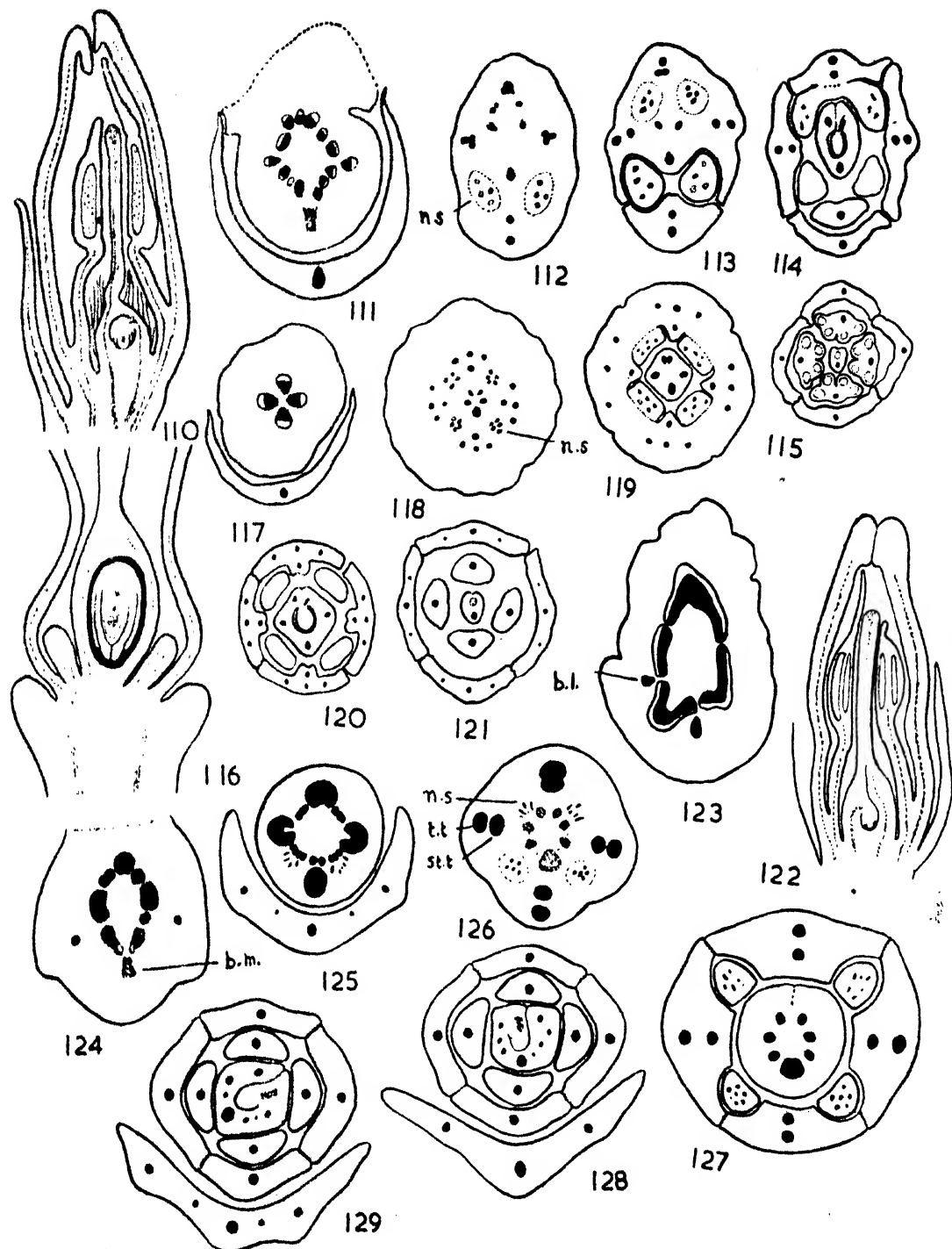
ENDOSPERM

The endosperm is of the nuclear type. It becomes cellular first around the embryo when the latter is a small globular mass (Fig. 174), by a process of indentation. In *Bellendena* and *Persoonia*, nuclear fusions occur in the antipodal endosperm resulting in polyploid nuclei (Fig. 177 and 213). The nucleus standing close to the postament is especially large and seems to be concerned in the nutrition of the embryo sac (Fig. 178). An exactly similar situation is noticed in some Sterculiaceae e.g., *Abroma augusta* and *Pentapetes phoenicea* (Venkata Rao, 1953). There is no aggressive enlargement of the embryo sac after fertilization in *Bellendena* and *Persoonia*, the digestion of the nucellus being slow and prolonged. The endosperm in the antipodal region in *Bellendena* remains nuclear for a long time. Ultimately the whole of the endosperm becomes cellular. The cells in the antipodal region contain large polyploid nuclei. The endosperm is even in outline and does not show papillate haustorial cells as are reported in *Macadamia ternifolia* (Kausik, 1938b).

Mature seeds of *Bellendena* are endospermic. The endosperm cells are packed with starch and other reserve materials. The number of layers of endosperm cells surrounding the embryo varies in the different parts of the seed, the endosperm

EXPLANATION OF FIGURES

Figs. 87-109. Figs. 87-98. *Symphyonema montanum*. Fig. 87. L. S. flower bud. Figs. 88-96 and 98. Sections at various heights through flower bud. Fig. 97. Section through the tube formed by filaments, and style. Figs. 99-109. *Agastachys odorata*. Fig. 99. L. S. flower bud. Fig. 100. T. S. peduncle showing origin of floral stele. Figs. 101-109. Sections through flower bud at various heights. Fig. 105. T. S. ovary at the level of attachment of ovule. Fig. 108. T. S. stigma. b.m.=bract midrib; b.l.=bract lateral; st.t.=staminal trace; t.t.=tepale trace. Figs. 87, $\times 25$; Figs. 88-96 and 98, $\times 40$; Fig. 97, $\times 120$; Fig. 99, $\times 25$; Figs. 100, 101, 103, 104, 106, 107 and 109, $\times 25$; Fig. 102, $\times 40$; Fig. 105, $\times 40$; Fig. 108, $\times 80$. (Explanation in text.)



Figs. 110-120.—Figs. 110-115. *Beauprea paniculata*. Fig. 110. L. S. flower bud. Figs. 111-115. T. S. at various heights through flower bud. Fig. 116-121. *Beauprea penchari*. Fig. 116. L.S. base of flower bud. Figs. 117-121. T. S. at various heights through flower bud. Figs. 122-129. *Cenarrhenes nitida*. Fig. 122. L. S. flower bud. Figs. 123-129. T. S. at various heights through flower bud. b.l.=bract laterals; b.m.=bract midrib; stt.=staminal trace; t.t.=tepal trace. Figs. 110-121, $\times 25$; Fig. 116, $\times 35$; Figs. 122-129, $\times 12$.

being thicker around the radicle than around the cotyledons (Figs. 196 and 197). In one young seed, though the embryo was normal, there was no trace of endosperm (Figs. 190 and 191). In *Persoonia*, the seeds are non-endospermic or only a trace of endosperm persists.

In *Cenarrhens nitida* the enlargement of the ovule after fertilization is sudden and pronounced. This results in a transverse rupture of the nucellus at about the middle of its height and sometimes the inner integument also becomes involved (Figs. 255 and 257). As the seed grows, a lenticular cavity develops which becomes filled with a clear nutritive fluid. As already described, there is a hypostase of elongated, thin-walled, glandular cells below the embryo sac which stands out distinctly from the large, isodiametric, scantily cytoplasmic, light staining cells constituting the rest of the nucellus. During seed development, the cells of the hypostase fall apart giving rise to an irregular space which serves to connect the antipodal end of the sac with the nucellar cavity (Figs. 258, 259 and 261). The endosperm is scanty and does not extend into the enlarging nucellar cavity. This cannot, therefore, be described as 'endosperm haustorium' in the strict sense. A somewhat similar condition is described by Jordaan (1916) in the S. African *Brabeium stellatifolium*. The endosperm in *C. nitida* remains nuclear even at the stage shown in Fig. 261. It is doubtful if it ever becomes cellular. The nucellus persists for sometime and functions as the perisperm. In the mature seed both the endosperm and perisperm become absorbed and the seed cavity is filled by the large succulent embryo (Figs. 268 and 269).

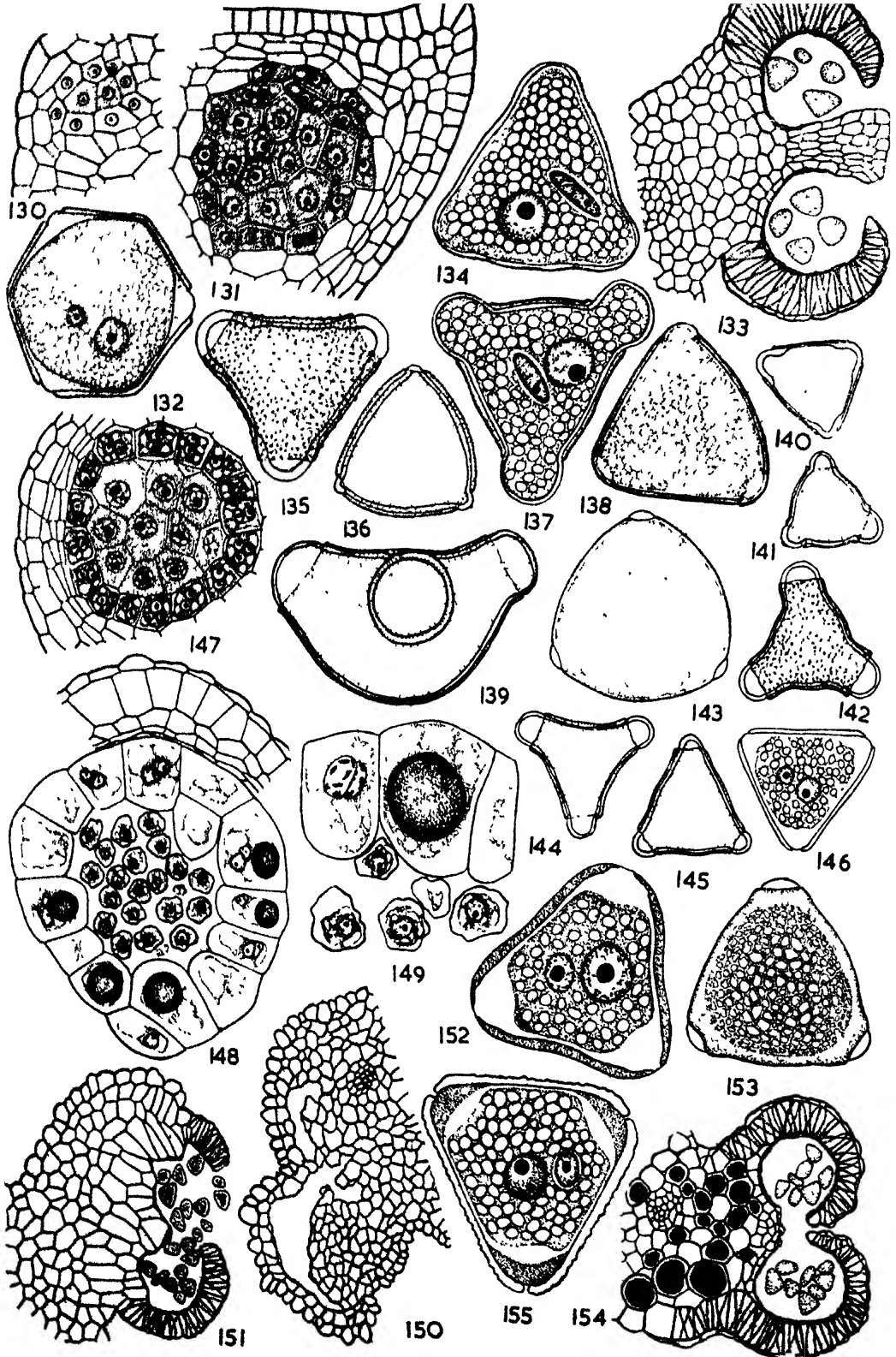
EMBRYO

The fertilised egg does not rest for a prolonged period. The first division of the oospore is transverse and results in the basal cell *cb* and the terminal cell *ca* (Fig. 262). The next division is longitudinal in both cells and results in a tetrad of cells in two tiers (Figs. 179, 180, 263 and 264). The derivatives of both *ca* and *cb* enter into the embryo proper, there being no suspensor (Figs. 181-185, 214, 234-236, 265-267). In *B. montana*, sometimes the lowest cell of the embryo presents a glandular appearance (Fig. 184), since this comes into intimate contact with the glandular nucellar cells, it seems to function in absorption of food materials. The embryo development in all Persooniaceae studied keys out to *Penaea* variation of Asterad type (Johansen, 1950).

The mature embryo in *Bellendena* and *Cenarrhens* shows a well developed radicle and two thick cotyledons. The cells of the embryo are packed with food materials. The cotyledons do not show the basal lobes that are characteristic of genera like *Grevillea* and *Hakea* (Fig. 198).

SEED AND FRUIT

The mature seed of *Bellendena* is fusiform and slightly flattened parallel to the fruit; it is 5-6 mm. long and c. 2 mm wide at the middle. In the mature ovule the outer integument is biseriate and the inner 4 cells thick (Fig. 201). After fertilization, both the integuments increase in thickness, the outer becoming 5 layered and the inner 6-7 cells thick (Fig. 202). The maximum thickness of the testa is attained in seeds with embryo just showing cotyledon primordia. The coats of mature seed are devoid of mechanical tissue. Several layers of both integuments break down (Fig. 203) and mature seed coats show only two layers: the innermost layer of tannin filled cells belonging to the testa and the palisade like layer of thin walled cells belonging to the tegmen (Fig. 204). The seed coats of *Cenarrhens* are also flimsy.



The style in *Bellendena* is persistent. Though it is at first terminal, as the ventral margin of the fruit grows more rapidly than the dorsal, it becomes bent and ultimately fits into a depression at the summit of the fruit (Figs. 192-194). The pericarp consists of 5-6 layers of thin walled parenchyma surrounded by strongly cutinised epidermal layers, there being no mechanical tissue (Fig. 200). The mature fruit is obovate, dry and light and apparently wind dispersed. A membranous wing develops to the outside of the ventral margin (Fig. 195). The fruit does not show any dehiscence mechanism; decay of or mechanical injury to the brittle pericarp seems to result in liberation of the seed.

In *Persoonia* the fruit is a small succulent drupe with persistent style (Fig. 215). After fertilization, the glandular cells lining the loculus proliferate and produce a tissue which completely envelops the seeds (Figs. 216 and 217). The ovary wall shows two zones: the outer of tannin filled cells which ultimately develops into the succulent epicarp and the inner zone and the tissue surrounding the seeds which form the stony endocarp.

In *Cenarrhnes*, the fruit is a purplish spherical drupe (Plate XVII, 5) The ovary wall shows two distinct regions from early stages (Figs. 47 and 253). The outer zone consists of scantily cytoplasmic, light staining cells; this develops into the succulent epicarp; the inner consisting of small richly protoplasmic cells develops into the stony endocarp (Figs. 268 and 269).

In *Agastachys* the fruit is a small 3-winged samara. Two larger wings develop to the outside of the median dorsals and a small wing to the outside of the dorsal bundle (Figs. 270 and 271).

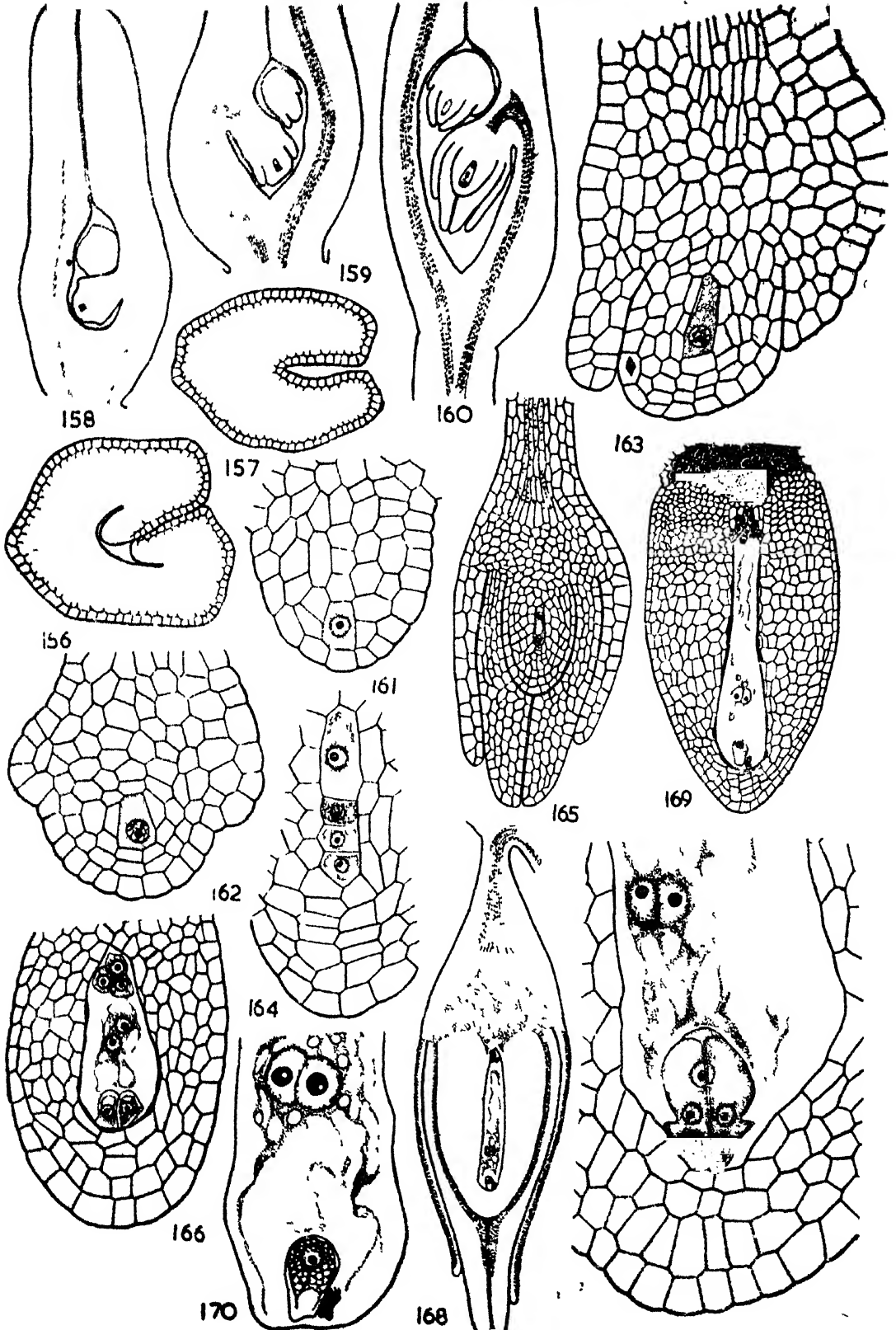
STERILITY

There is good fruit set in *Bellendena*; an inflorescence contains 50-80 flowers of which 50 per cent or more give rise to fruits (Plate XVII, 2). In *Cenarrhnes* and *Agastachys* there is relatively less fruit set (Plate XVII, 5) and sometimes all the flowers of an inflorescence wither away without forming a single fruit. Sometimes in apparently normal fruits, the seed cavity was found to be empty.

In *Bellendena* and *Persoonia*, though the carpel is 2-ovulate the fruits are commonly 1-seeded, 2-seeded ones being very rare. In *Bellendena* both the ovules become fertilised and start development as seeds. Later, in several cases the two seeds were seen to become fused by webbing together of their integuments. The rapid elongation of one seed results in dislodging the other and therefore to the degeneration of the latter. Shrunken remnants of such seeds are often found attached to the functional seeds (Figs. 175 and 194). However, in some other cases seeds are seen to degenerate without any apparent cause. The process of degeneration may start even at a late stage when the seed is showing a large globular embryo. In one young seed which was still attached to the placenta, the integuments and nucellus were showing signs of degeneration but the endosperm was

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Figs. 130-155.—Microsporogenesis and male gametophyte in Persooniaceae. Figs. 130-132. *Cenarrhnes nitida*. Figs. 133-145. *Persoonia* sp. Figs. 133 and 134. *P. juniperina*. Fig. 135. *P. salicina*. Fig. 136. *P. saundersiana*. Fig. 137. *P. virgata*. Figs. 138 and 139. *P. lanceolata*. Fig. 140. *P. linearis*. Fig. 141. *P. myrtilloides*. Fig. 142. *P. ferruginea*. Fig. 143. *P. saccata*. Fig. 144. *P. oxyrocooides*. Fig. 145. *P. microcarpa*. Fig. 146. *Beauprea paniculata*. Figs. 147-152. *Bellendena montana*. Figs. 148-150. Sections through pollen sterile anthers. Figs. 153. *Symphyonema paludosum*. Figs. 154 and 155. *Agastachys odorata*. Figs. 130, 131, 134, 137, 143, 147, and 148, $\times 270$; Figs. 132, 133, 138, 139, $\times 600$; Figs. 136, 140, 141, 142, 144, 145, $\times 335$; Figs. 135, 149, $\times 480$; Figs. 146, $\times 720$; Figs. 150, 151, $\times 120$; Figs. 152, 153, 155, $\times 1200$. Fig. 154, $\times 100$.



showing nuclear divisions (Figs. 187-189). The endosperm in this case is probably parasitising on the sporophytic tissues. In *Persoonia* sp. the integument of the functionless ovule was found fused to the false septum (Fig. 233). The enlargement of the fruit probably resulted in breaking off the funicle from the placenta and therefore led to the degeneration of the seed

DISCUSSION

The present studies support Engler's (1894) conclusion that the *Persoonieae* are the most primitive tribe of the *Proteaceae*. The members show a large number of primitive morphological, floral anatomical, embryological and cytological features. However, a comparative study of the different genera shows that some evolution has taken place within the tribe. These evolutionary trends are discussed in the following pages.

The members studied show evolution in vegetative features. *Cenarrheneae* and *Agastachys* are confined to the rainforests or their fringes in Tasmania. Though usually shrubby, they attain the size of trees on the west coast. Some species of *Persoonia* (e.g. *P. longifolia*) are trees and others shrubby. Species of this genus show wide range and inhabit diverse situations from sea level to c. 2 000' altitude. Both species of *Symphyonema* are undershrubs endemic in New South Wales. *Bellendena montana* is a gregarious shrub which inhabits sub-alpine meadows of Tasmania above 3,500' altitude. The climate in such places is cool and moist throughout the year and the soil is covered by snow in winter months.

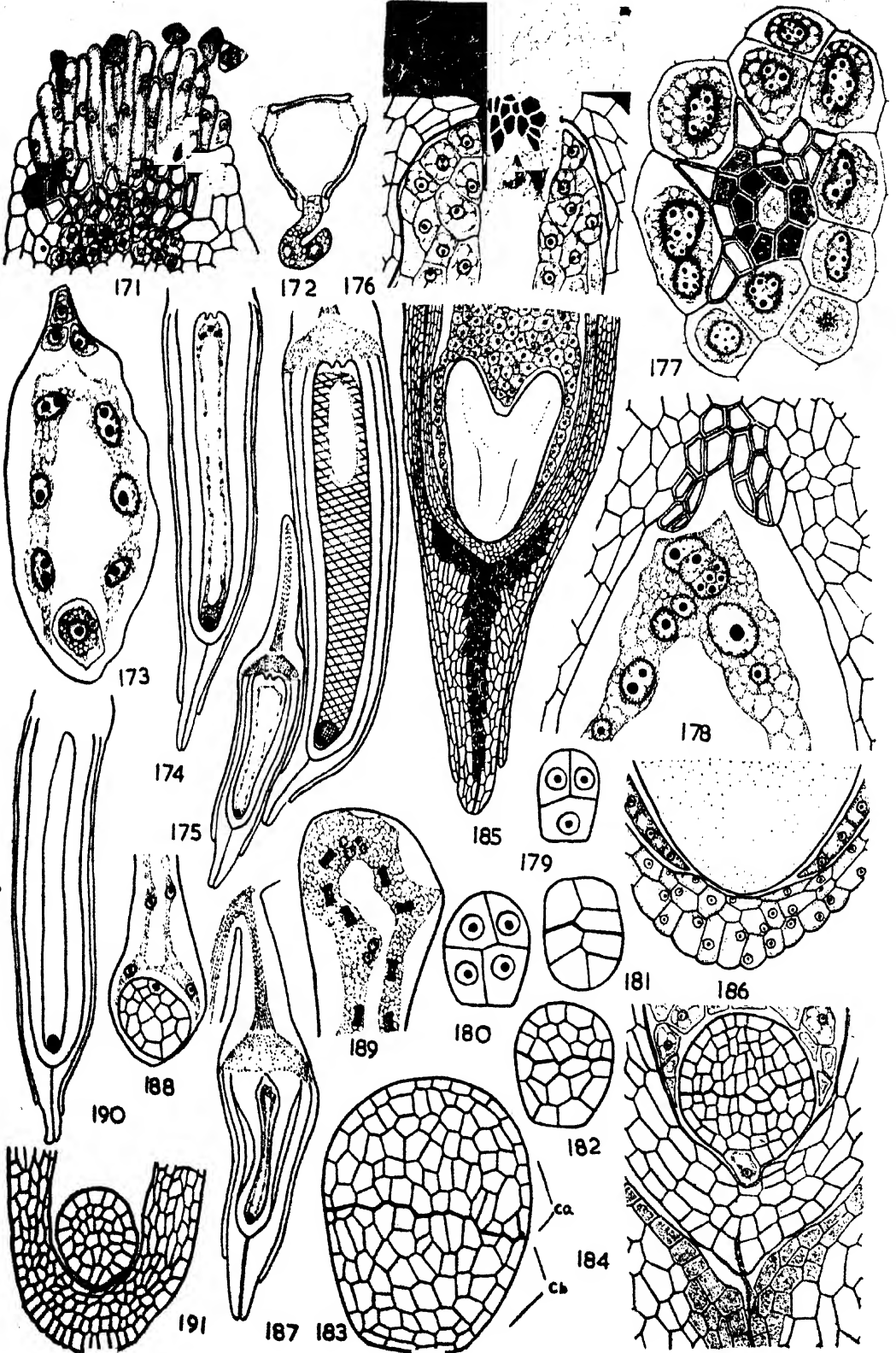
The leaves in *Symphyonema* are 2-3 times pinnatisect (Fig. 42). They are palmately 3-lobed, 3-traced, somewhat succulent and externally veinless in *Bellendena* (Figs. 20 and 21), expanded, simple, and dentate in *Cenarrheneae* and *Beauprea* and succulent and entire in *Agastachys*. In *Persoonia* they range from acicular (*P. succata* and *P. pinifolia*) to oblong-lanceolate form (*P. lanceolata* and *P. toru*). In several species they show xeromorphic features: the edges of the narrow, small, spine tipped leaves of *P. juniperina* are held vertically (cf. Plates XVII and XVIII).

In several species of *Persoonia* the flowers are solitary axillary and diffusely scattered. *P. pinifolia* shows the evolution of inflorescence (and bract) by the aggregation of flowers towards the ends of branches. The leaves in this region are acicular like the vegetative leaves but smaller and bract-like in appearance. In *Beauprea* and *Symphyonema* the inflorescence is a lax panicle. It is more condensed in *Cenarrheneae* and *Agastachys* due to the suppression of the peduncles. The bracts in these genera are relatively large and persistent.

The ebracteate, pedicellate, regular, glandless flowers of *Bellendena* with stamens completely free from the tepals, and stipitate ovary seem to represent the most primitive floral structure. Evolution is noticed in the different members in the suppression of pedicel, introduction of a bract and its persistence, adnation of tepals and stamens, connation of filaments, suppression of the appendage of the

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Figs. 156-170. *Bellendena montana*. Figs. 156 and 157. T. S. through young ovary and style. Figs. 158-160. L. S. through ovaries at different stages of development. Fig. 161. Ovule primordium with archesporium. Figs. 162 and 163. Ovules with megaspore mother cell. Fig. 164. Nucellus with linear tetrad. Fig. 165. L. S. ovule with 2-nucleate embryo sac. Fig. 166. Nucellus with young embryo sac. Fig. 167. Micropylar part of mature embryo sac. Fig. 168. Ovule with mature embryo sac. Figs. 169 and 170. Nucellus and micropylar part of embryo sac from above. Figs. 156-158, $\times 100$; Figs. 159 and 160, $\times 75$; Figs. 161, 164 and 170, $\times 360$; Figs. 165 and 168, $\times 180$; Fig. 166, $\times 270$; Fig. 167, 600; Fig. 169, $\times 180$.



stamen and stipe of the ovary, development of vascularised nectary, tendency towards zygomorphy of the flower and attainment of partial male sterility. The ancestral carpel seems to be multiovulate, a condition still found in *Garnieria* (Persoonieae) and in other tribes of the family viz., Placospermeae, Embotrichae and Telopeae. The abnormal multiovulate carpel noticed in *Persoonia saccata* seems to be atavistic. The 2- and 1-ovulate conditions in other Persoonieae seem to be derived by suppression of the extra ovules. Evidence for the suppression of the second ovule is found in *Cenarrhenes* in the shape of a vestigial ovule on the sterile carpellary margin and in the occasional development of a normal second ovule. In *Agastachys*, the second ovule as well as the marginal bundle feeding it are completely suppressed. The fruits in *Bellendena* do not show any mechanical tissue or dehiscence mechanism or any special adaptations for dispersal. In *Agastachys* the fruit is a small samara adapted for wind dispersal, while in *Persoonia* and *Cenarrhenes* they are attractive drupes adapted for bird dispersal.

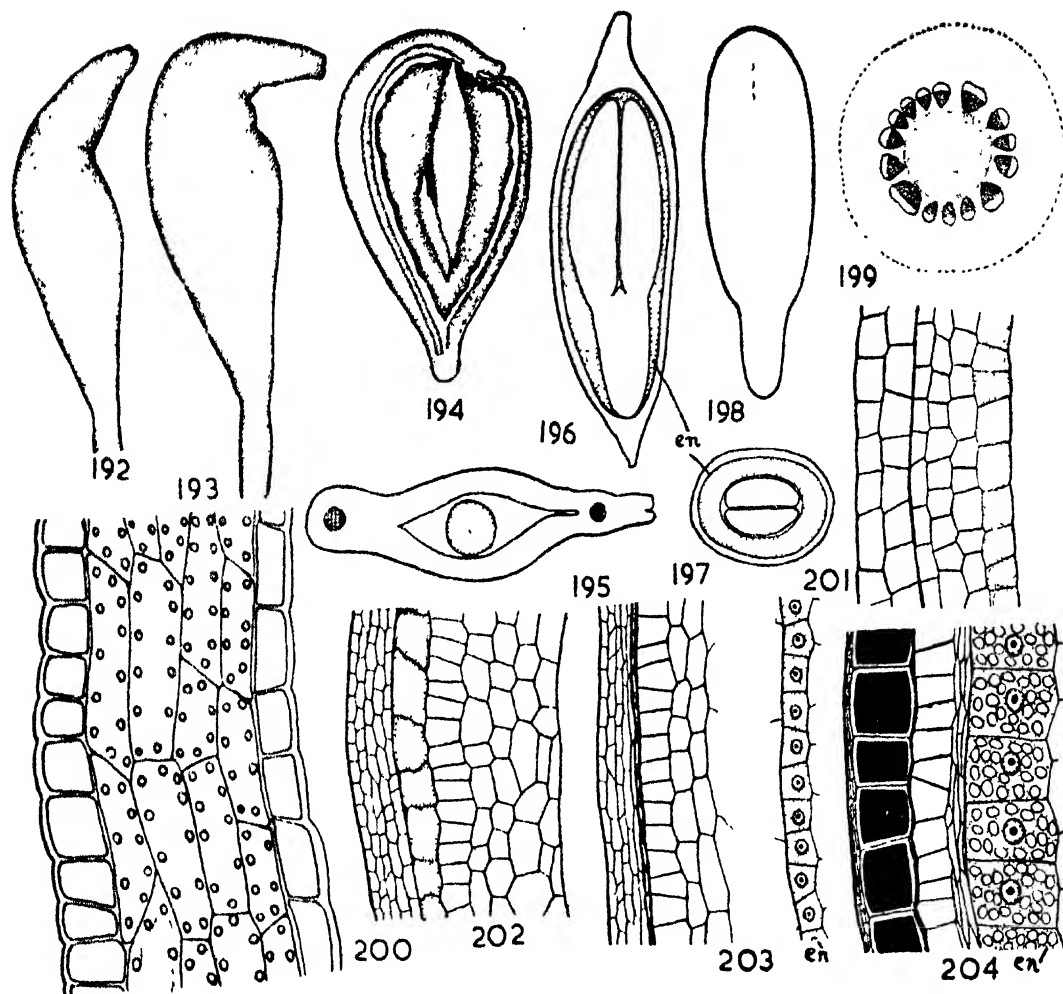
In general there is an elaboration of floral stele by an increase in carpellary traces and intercalation of vascular supply for the nectary, though reduction is also noticed in suppression of tepal marginals or one ventral bundle in *Agastachys*. The stamens become adnate to the tepals but the union does not involve their traces. The organisation of the staminal traces as a pair of bundles to the inside of the tepal midrib is interesting. A similar antitepalous position and twin bundled staminal trace are noticed in *Helicanthes elastica* (Johri, Agrawal and Garg, 1957) and *Nuytsia* sp. (Narayana, 1958) of Loranthaceae. The significance of this peculiar staminal trace will be discussed later.

The 3-traced carpel noticed in *Bellendena* and *Beauprea* sp. seems to be primitive and the 5- and 7-traced carpels of *Persoonia* and *Cenarrhenes* seem to be derived by an elaboration as suggested by Eames (1931).

The morphology of the perianth is linked with that of the nectary. If the nectary could be homologised to the corolla of a dichlamydeous flower, the perianth could be equated to the calyx. The 4 alternitpalous lobes of the nectary seen in *Persoonia*, *Cenarrhenes* and *Beauprea* offer a tempting comparison to the petals of a dichlamydeous flower. But in S. African *Brabeium*, (and *Macadamieae*) the lobes of the nectary unite to form a cup situated between the tepal-stamen whorl and the pistil and not in the position of the corolla of a dichlamydeous flower. In *Grevilleae* and *Telopeae* the lobes of the nectary are not only connate but the nectary becomes zygomorphic due to the suppression of one or two anterior lobes. If the nectary is to be homologised to the corolla, then it must be conceded that apetaly (cf. the glandless flowers of *Bellendena*, *Symphyonema* and *Agastachys*), polypetaly, gamopetaly and zygomorphy of the corolla have been attained within the family. The nectary is non-vascular in *Oriteae* and *Banksia* sp. In the Persoonieae it is vascularised by strands derived either from tepal laterals or staminal traces or outer margins of intertepalous sectors of floral stele. In no

EXPLANATION OF FIGURES

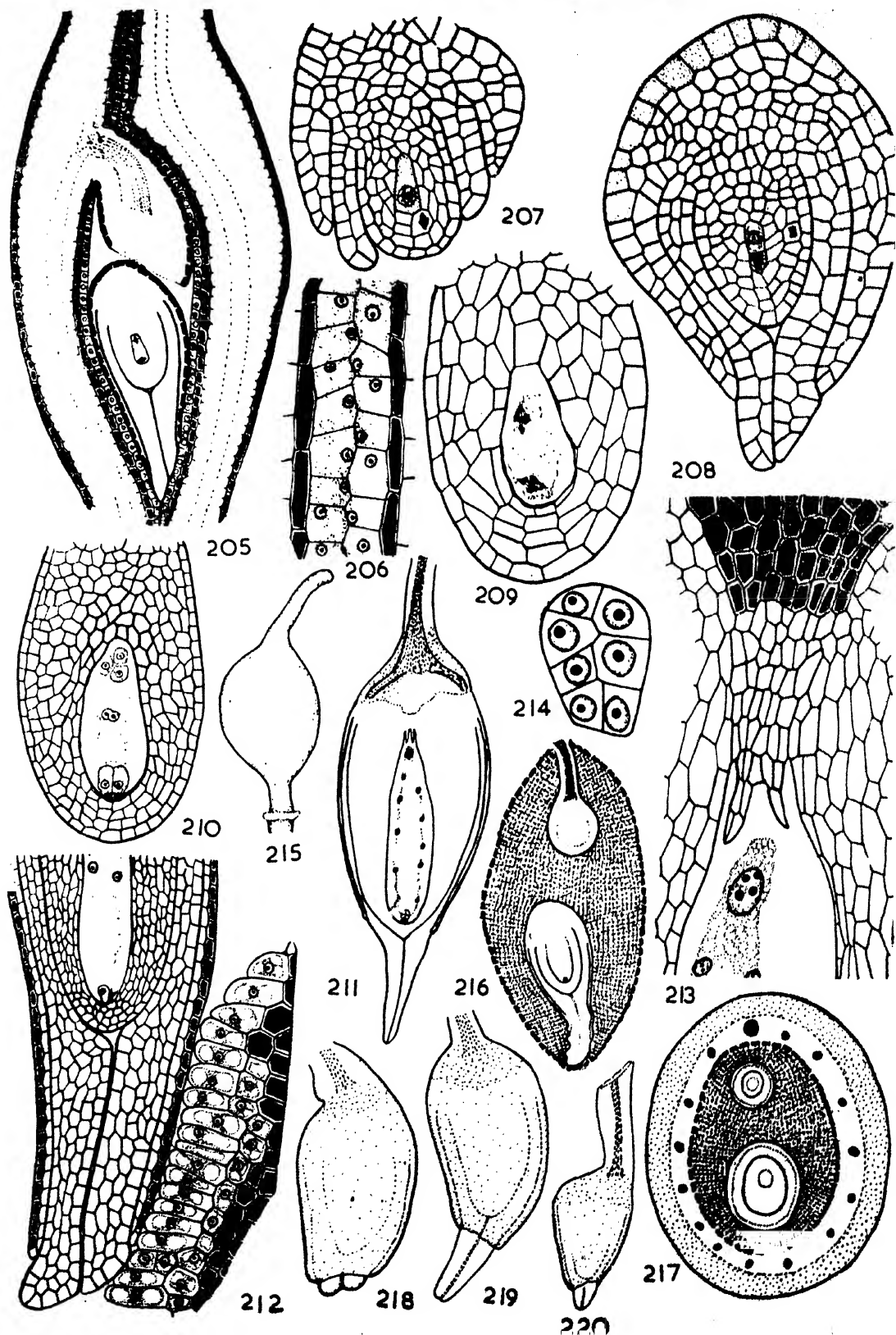
FIGS. 171-191.—Endosperm and embryo development in *Bellendena montana*. Fig. 171. Stigmatic hairs and germinating pollen grains. Fig. 172. A germinating pollen grain. Fig. 173. A fertilised embryo sac. Figs 174 and 175. L. S. developing seeds. Fig. 176. L. S. part of seed showing antipodal endosperm and postament. Fig. 177. T. S. postament. Fig. 178. L. S. postament and some endosperm. Figs. 179-185. Stages in the development of the embryo. Fig. 186. Tip of radicle and some endosperm and nucellar cells. Fig. 187. L.S. degenerating seed. Figs. 188 and 189. Micropylar and antipodal parts of embryo sac from the above. Fig. 190. L. S. abnormal seed devoid of endosperm. Fig. 191. Micropylar part of the above. Fig. 171, $\times 130$; Fig. 172, $\times 620$; Figs. 173 and 177, $\times 270$; Figs. 174 and 175, and 190, $\times 30$; Figs. 176, 184, 186, 188, 189, and 191, $\times 160$; Figs. 178, $\times 600$; Figs. 179-183, $\times 210$; Figs. 185 and 187, $\times 60$.



FIGS. 192-204. - *Bellendenia montana*. Figs. 192-194. Developing fruits. Fig. 195. T. S. fruit. Fig. 196. L. S. seed. Fig. 197. T. S. seed. Fig. 198. Entire embryo. Fig. 199. T. S. chalazal part of the seed showing ring of strands formed by division of ovular trace. Fig. 200. Section of pericarp. Fig. 201. T. S. integuments of mature ovule. Figs. 202-204. T. S. of coats of developing and mature seeds. Figs. 192, 193 and 195, $\times 10$; Fig. 194, $\times 4$; Figs. 196-198, $\times 6$; Figs. 199 and 200, $\times 75$; Figs. 201-204, $\times 340$.

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FIGS. 205-220. *Persoonia* sp. Figs. 205-217. *P. juniperina*. Fig. 205. L. S. ovary. Fig. 206. Transmitting tissue of style. Fig. 207. Section of ovule with megaspore mother cell. Fig. 208. Section of ovule with megaspore tetrad. Fig. 209. Nucellus with developing embryo sac. Fig. 210. Nucellus with young embryo sac. Fig. 211. L. S. young seed showing postament. Fig. 212. L. S. micropylar part of fertilised ovule and adjacent glandular obturator. Fig. 213. L. S. antipodal part of embryo sac showing postament and some endosperm. Fig. 214. A young embryo. Fig. 215. A fruit. Figs. 216 and 217. L. S. loculus and T. S. fruit showing tissue developed from glandular cells of locular epidermis. Figs. 218-220. Entire ovules of *P. toru*, *P. microcarpa* and *P. saundersiana* respectively. Fig. 205, $\times 60$; Figs. 206-210, 212-214, $\times 160$; Fig. 211, 216 and 217, $\times 25$; Fig. 215, $\times 3$; Fig. 218, $\times 35$; Fig. 219, $\times 60$; Fig. 220, $\times 25$.



case is it fed by independent traces which cause gaps in the floral stele like those of tepals. Therefore, neither the position, nor the morphology nor the vasculature of the nectary give any evidence of its homology to the corolla. It seems to be only a glandular nectar secreting outgrowth of the thalamus. Similar outgrowths are also found in some other monochlamydeous and dichlamydeous families. Since the nectary cannot be homologised to the corolla of a dichlamydeous flower, the perianth cannot be equated to the calyx. The flower seems to be primitively monochlamydeous and the perianth parts are best designated as tepals.

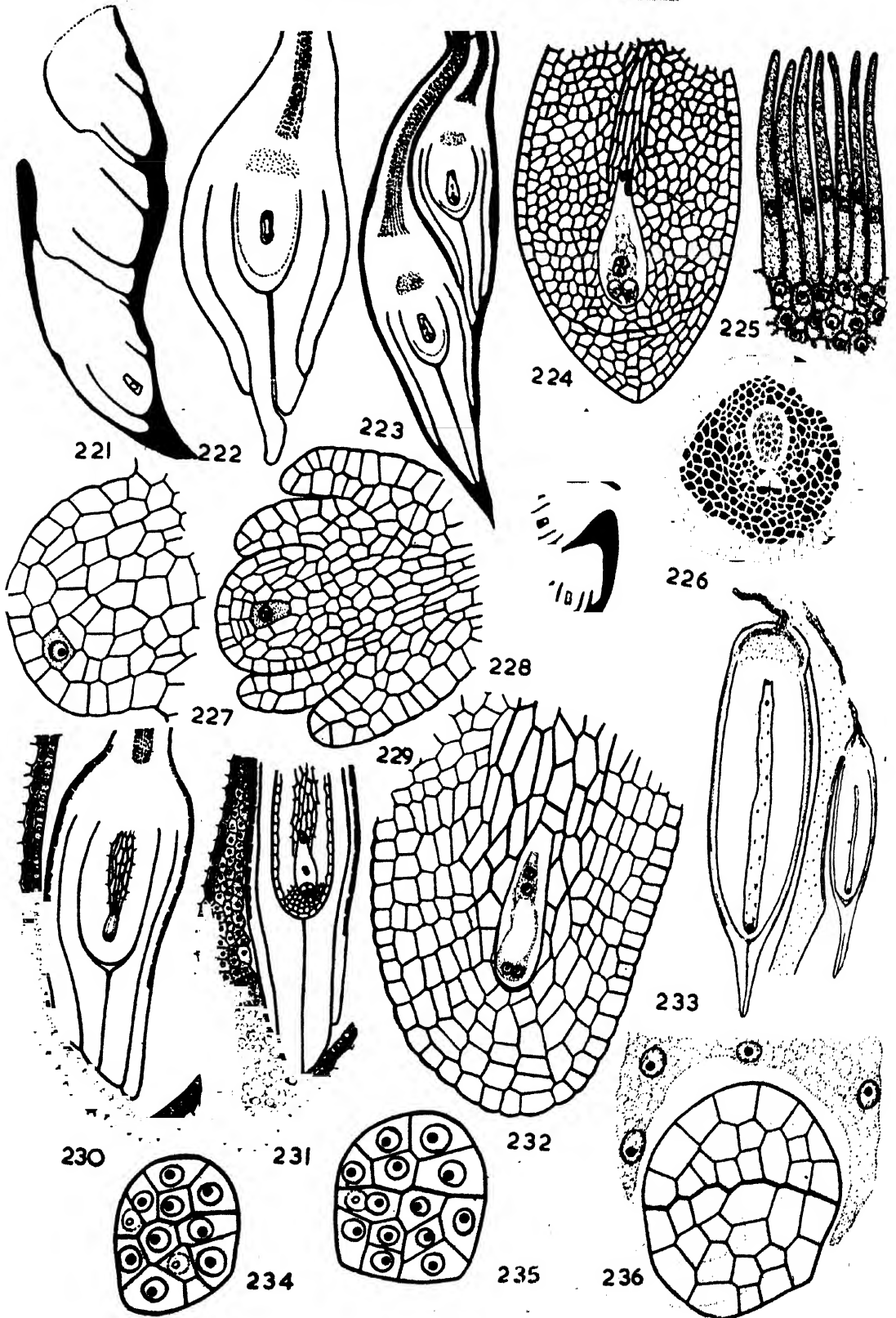
The Persoonieae show close resemblance in embryological features : 5-6 layered anther wall with hypodermal endothecium and tapetum of the secretory type, 3-porate, triangular, 2-celled pollen, transmitting tissue in the style, crassinucellate, bitegmic ovules in which the inner integument is more massive than the outer, an elongated micropyle formed by the inner integument, branching of the funicular vascular bundle in the chalaza, *Polygonum*-type of embryo sac development, fusion of the polar nuclei at the time of fertilization, inconspicuous antipodals nuclear type of endosperm, development of the embryo according to *Penaea* variation of Asterad type, and flimsy seed coats. Evolution is noticed in the reduction of the length of the funicle and the lateral attachment of the ovule, development of special devices which facilitate the progress of pollen tubes (obturator), and tendency towards the formation of 'endosperm' haustorium. *Bellendera* is the only member in which the seeds are endospermic; in other Persoonieae as well as in the rest of the family they are non-endospermic.

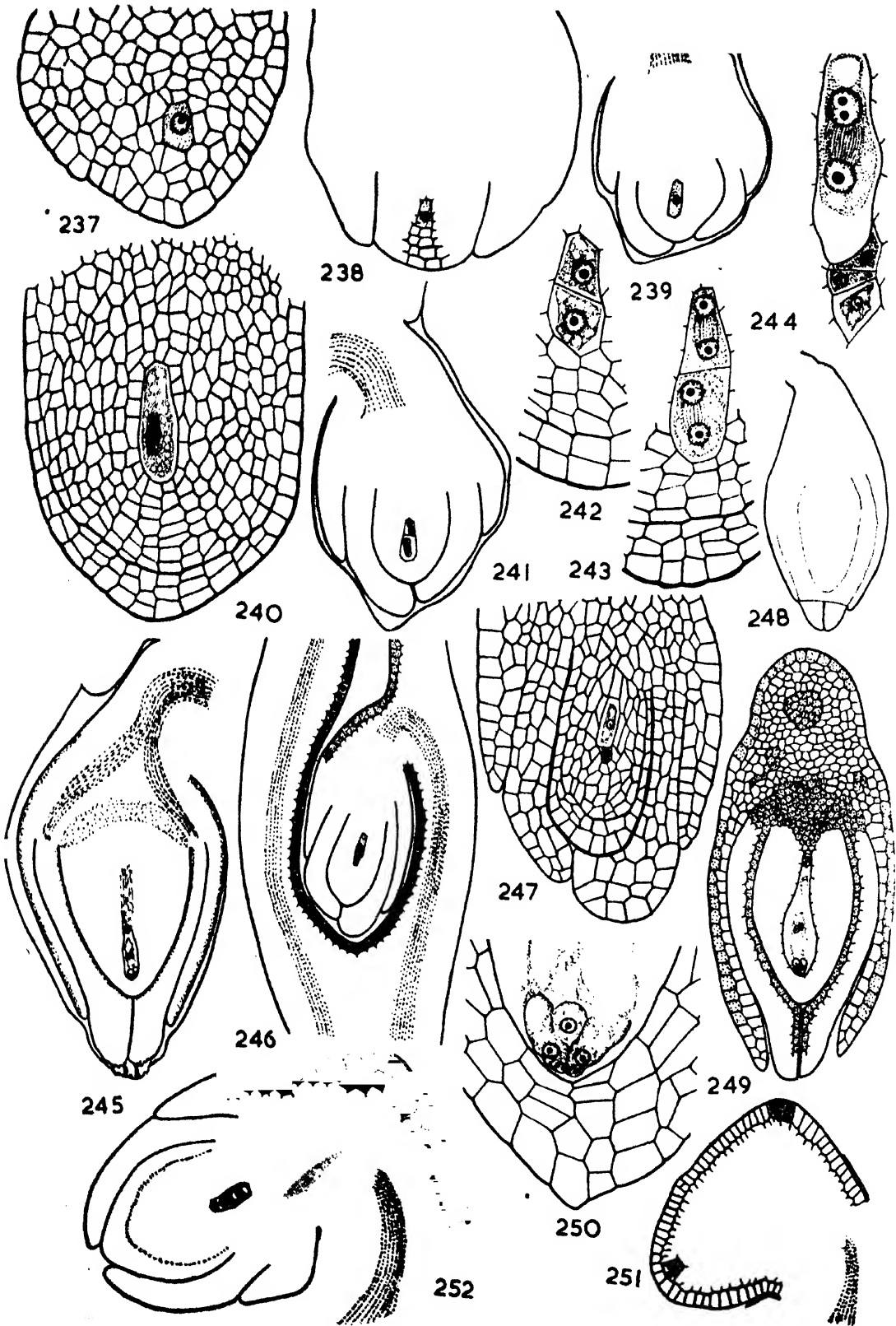
As is commonly noticed in other angiosperms, evolution has not progressed at the same rate in all floral organs in any genus or species. In *Picnostylis* section of *Persoonia*, for example, zygomorphy of the flower has been attained due to the development of a saccate perianth and curved style, but the flowers retain primitive features in the presence of pedicel, fusion of the twin bundles of the staminal trace at a relatively high level, conspicuous vascularised appendage for stamen and stipe for the ovary.

Several of the evolutionary tendencies noticed in the Persoonieae become established and accentuated in other tribes of the family and form their important characteristics, e.g., adnation of stamen and tepal culminates in the complete union of their traces in Oriteae; connation of filaments results in connation of the anther lobes in Conospermeae; connation of the lobes of the nectary leads to its zygomorphy in Grevilleae, Telopeae and Embothrieae; curved style and lateral stigma lead to the development of a pollen collecting apparatus in *Spatalla* of Proteaceae. Grevilleae Telopeae and Embothrieae; zygomorphy of the flower becomes accentuated in Grevilleae, Telopeae and Embothrieae partial male sterility in Conospermeae, torsion of the ovary in the Oriteae and development of a well defined endosperm haustorium in several genera of the Grevilloideae.

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FIGS. 221-236.—*Persoonia* sp. Figs. 221-226. *P. saccata*. Fig. 221. L. S. abnormal ovary with 3 ovules. Fig. 222. L. S. ovule with 2-nucleate embryo sac. Fig. 223. L. S. loculus of mature ovary showing attachment of the ovules (from 2 sections). Fig. 224. Nucellus with mature embryo sac. Fig. 225. Glandular stigmatic hairs. Fig. 226. T. S. style with transmitting tissue. Figs. 227-229. *P. gunnii*. Fig. 227. Ovule primordium with archesporium. Fig. 228. Loculus of ovary. Fig. 229. L. S. young ovule with megaspore mother cell. Figs. 230-232. *P. lanceolata*. Fig. 230. L. S. young ovule with adjacent part of the loculus showing glandular epidermal cells. Fig. 231. L. S. mature ovule and glandular obturator; Fig. 232. Nucellus with 4-nucleate embryo sac. Figs. 233-236. *P. pinifolia*. Fig. 233. L. S. part of fruit with a normal and a degenerating ovule. Figs. 234-236. Stages, in development of the embryo; Figs. 221, 222 $\times 75$; Figs. 223, 226, 228 $\times 35$; Figs. 224, 225, 229, 232 $\times 160$; 227 $\times 270$; Figs. 230, 231, $\times 60$; Fig. 233, $\times 6$; Figs. 234-236, $\times 360$.





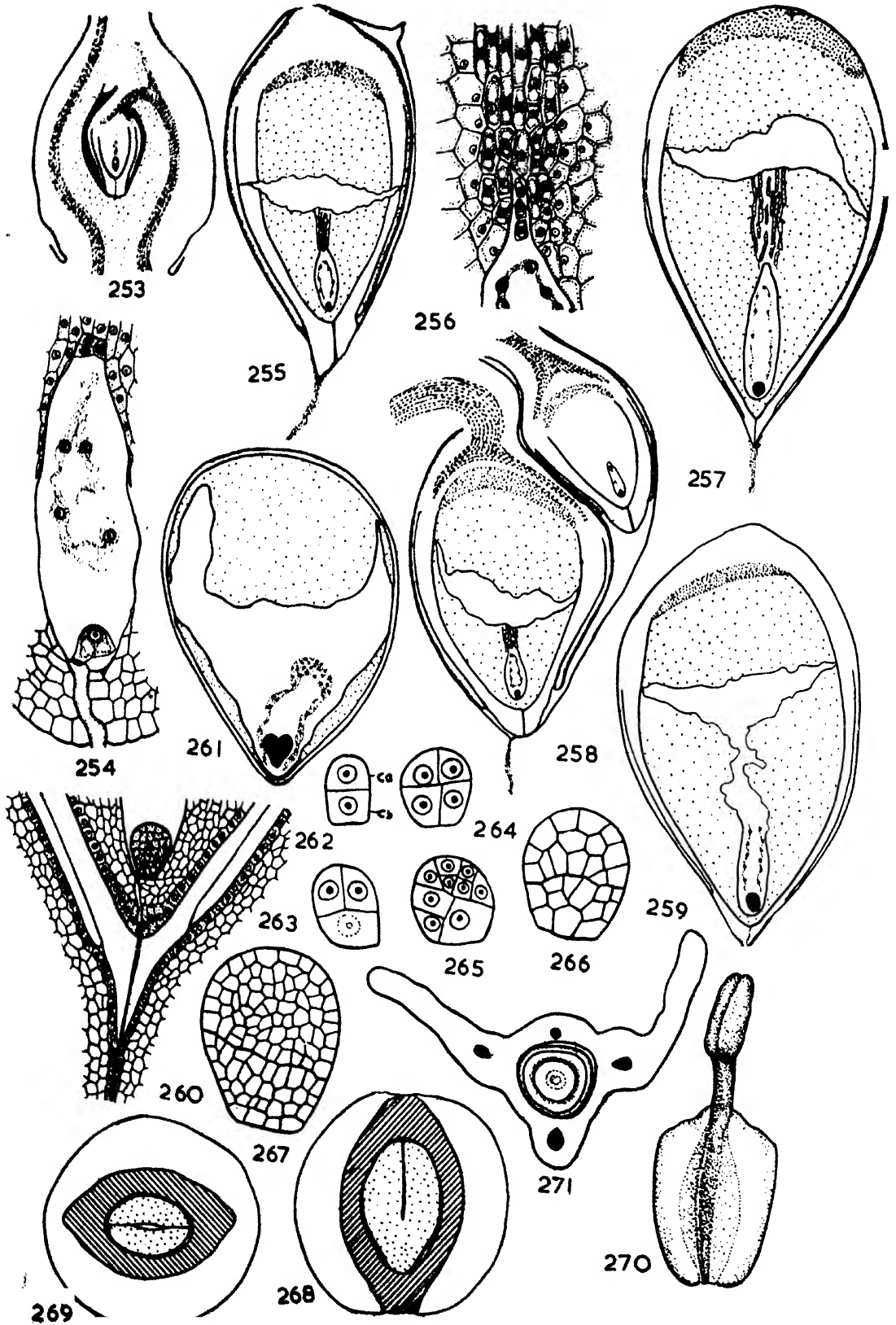
The sum of morphological, floral anatomical and embryological evidence points to *Bellendena* being the most primitive member of the Persoonieae which probably escaped extinction by continuing to inhabit situations similar to those under which it originally evolved. Australia and adjacent islands did not experience the drastic effects of glaciation and several examples of the most primitive fauna and flora persist there till today, e.g., the fresh water shrimps of Tasmania, the egg laying mammals of Australia. Five of the nine vesselless angiosperms are endemic in New Guinea and New Caledonia (Bailey, 1949). *Bellendena* may be another such palaeo-endemic.

Lancaster (1952) thought that 7, the haploid chromosome number of *Persoonia* represents the basic number in the family and that $n=14, 13, 12, 11$ and 10 noticed in other genera of the family represent reduction series derived by stepwise loss of one chromosome. The presence of $n=10$ in highly evolved genera like *Grevillea* and *Hakea* was thus explained but the same number in *Symphyonema* (Persoonieae) could not be accounted for. The writer, however, feels that the cytological data lend support to the conclusions drawn from morphological, floral anatomical and embryological evidence that evolution within the family has been progressive.

Bellendena ($n=5$) and *Persoonia* ($n=7$) have the smallest chromosome numbers in the family. The karyotypes of the two genera are very simple and closely similar with long and thick chromosomes which show median or sub-median constrictions and no trabants. The two genera show close resemblance also in the formation of a postament during seed development, structure of the endosperm with nuclear fusions in the antipodal region, presence of a large polyploid nucleus close to the postament and also the causes which lead to the sterility of the functionless seed. These are the only diploid genera in the family. There seem to be two possibilities for the derivation of their chromosome numbers: $n=5$ might have been derived from $n=7$ by loss of two chromosomes or $n=7$ might have been derived from $n=5$ by the addition of two chromosomes. The latter alternative seems to be more probable since at the diploid level addition of chromosomes is more favoured in nature. Moreover, *Persoonia* shows several features which prove that it is the derived genus, viz., a vascularised nectary, adnation of tepals and stamens, suppression of the produced connective (*Amblyanthera* section), tendency towards zygomorphy of the flower (*Picnostylis* section), tendency towards the suppression of the second ovule, non-endospermic seed and a specialised drupaceous bird dispersed fruit. Though some of the simpler morphological features (e.g., absence of the nectary) can be interpreted as being due to reduction, there are others which are generally agreed upon as being irreversible e.g., adnation of stamen and tepal, tendency towards zygomorphy of the flower and non-endospermic seed. *Persoonia*, therefore seems to be the derived genus. The increase in chromosome number in this genus seems to have led to great speciation, adaptability and tolerance capacity to diverse ecological conditions. While five out of the nine genera of Persoonieae are monotypic endemics, *Persoonia* with 72 species shows wide range and variety in habitat. In fact it is the only living genus of Proteaceae which is represented in East and West Australia, Tasmania and New Zealand.

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Figs. 237-252.—Figs. 237-245. *Cenarrhenes nitida*. Figs. 237, 238, 239 and 241. L. S. through ovules at various stages of development. Fig. 240. Nucellus showing formation of megaspore dyad. Figs. 242 and 243. Formation of linear tetrad of megaspores. Fig. 244. Formation of 2-nucleate embryo sac. Fig. 245. L. S. ovule with mature embryo sac. Figs. 246-250. *Agastachys odorata*. Fig. 246. L. S. ovary with young ovule. Fig. 247. L. S. ovule with 1-nucleate embryo sac. Fig. 248. Entire ovule. Fig. 249. L. S. mature ovule. Fig. 250. Micropylar part of the embryo sac and the overlying nucellar cells. Figs. 251 and 252. L. S. ovules of *Beauprea paniculata*. Figs. 237, 240, $\times 270$; Figs. 238, $\times 135$; Figs. 239, 241, 249, $\times 85$; Figs. 242, 243, 250, $\times 360$; Fig. 244, $\times 600$; Figs. 245, 246, and 248, $\times 60$; Fig. 247, $\times 160$; Figs. 251 and 252, $\times 600$.



Bellendena and *Persoonia* seem to provide two types of floral structure (one without and one with nectary) and also two basic chromosome numbers. As in most angiospermous families, polyploidy and aneuploidy led to the evolution of the genera and tribes. This is accompanied by a diminution of chromosome size which is also noticed in other angiosperms e.g., diploid and polyploid species of *Dianthus*. *Symphyonema* ($n=10$) and *Cenarrhene* ($n=14$) seem to be tetraploids, on bases 5 and 7 respectively; $n=13$ of *Agastachys* seems to be a hypoploid on $n=14$. It is interesting to notice that in the last genus reduction in floral structure is found in association with reduction in chromosome number. Several physiological changes are known to accompany polyploidy (Muntzing, 1935; Stebbins, 1940; Blakeslee, 1941). The presence of a specialised method of nutrition in the shape of an 'endosperm' haustorium in *Cenarrhene* seems to be one such.

A comparative study of the Australian and extra-Australian Persooniaceae brings to light several points of interest. The genus *Cenarrhene* is common to Tasmania and New Caledonia, and *Persoonia* between Australia (including Tasmania), and New Zealand. There is close resemblance between *Cenarrhene nitida* (Tasmania) and *Beauprea* (New Caledonia) in morphological and floral anatomical features. The elongated tapering connective of the posterior stamen is strikingly similar in both genera. This feature is also shared by *Persoonia falcata* (E. Australia). These points show that the Australian, New Caledonian and New Zealand Persooniaceae must have had common ancestry. The discontinuous distribution of *Persoonia* and *Cenarrhene* can be ascribed to ancient dispersals by birds (as both have succulent drupes), and/or to the existence in the past, of land connections between the land masses which are now separate. Geological history shows that they formed a continuous continent in ancient times and that the islands have broken asunder and drifted apart since Cretaceous (Carey, 1938, 1955).

Levy (1958) suggested that the monotypic S. African *Brabeium stellatifolium* might belong to the Grevilloideae, placing too much stress on the whorled leaves. One important feature which distinguishes the Grevilloideae from the Persoonioideae is the occurrence of the flowers regularly in pairs in bract axils. In *Brabeium* the flowers occur in groups. This feature is never noticed in Grevilloideae but is found in some Persoonioideae viz., *Paranomus* and *Spatallopsis* of S. African Proteaceae. The stamens in *Brabeium* are described as being free from tepals; this feature is noticed only in *Bellendena* of the Persooniaceae. *Brabeium*, therefore seems to belong to Persooniaceae in which Engler had placed it. The genus resembles other Persooniaceae in the presence of 3-porate pollen, 2 orthotropic pendulous ovules and their structural features, embryo without suspensor, non-endospermic seeds and flimsy seed coats. The 'endosperm' haustorium of *Brabeium* is also closely similar to that of *Cenarrhene* (data from Jordaan, 1946). However, *Brabeium* shows advance in the connation of the lobes of the nectary into a cup. The haploid chromosome number of 14 in *Brabeium* (Darlington and Wylie 1955) is of particular interest since it coincides with that of *Cenarrhene* (Tasmania). The close similarity between S. African and Australian Persooniaceae points to their common ancestry. The

EXPLANATION OF FIGURES

FIGS. 253-271.—Figs. 253-269. *Cenarrhene nitida*. Fig. 253, L. S. mature ovary. Fig. 254. A fertilised embryo sac and adjacent cells. Fig. 255-261. L. S. young seeds in different stages of development. Fig. 258. L. S. loculus with two normally developed ovules. Fig. 260. Micropylar part of young seed and adjacent cells of loculus. Fig. 262-267. Stages in development of the embryo. Figs. 268 and 269. L. S. and T. S. of fruit. Figs. 270 and 271. Entire fruit and T. S. fruit of *Agastachys odorata*. Figs. 253 and 261, $\times 10$; Figs. 254 and 256, $\times 215$; Figs. 255 and 258, $\times 35$; Figs. 257 and 259, $\times 35$; Fig. 260, $\times 100$; Fig. 261, $\times 3$ Figs. 265, $\times 75$; Figs. 262-266, $\times 360$; Fig. 267, $\times 240$; Figs. 268, 269 and 270, $\times 4$; Fig. 271, $\times 15$.

probable causes which led to the distribution of the ancestral stocks between the two continents, now widely separated, will be discussed later.

The floral structure and chromosome number in *Dilobeia* (Madagascar) might prove useful in throwing some light on the nature of the ancestral stock that entered this land mass.

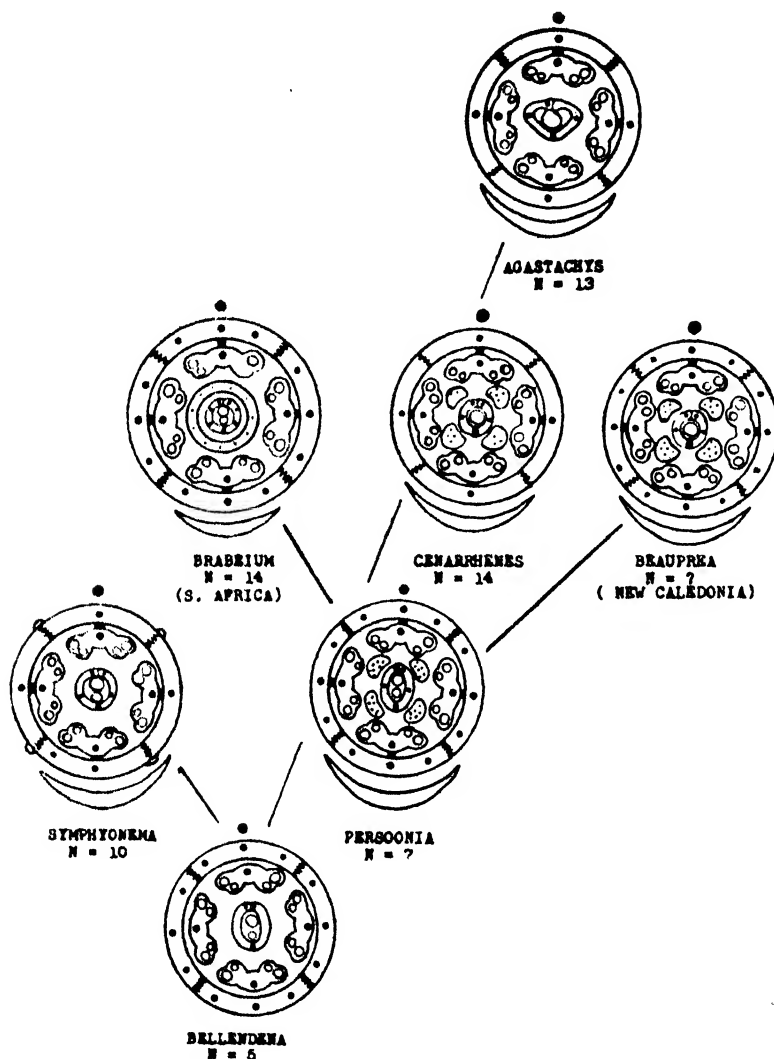


FIG. 272.—Evolution within the tribe Persoonieae according to the views of the writer.

The region which shows the greatest concentration of species is generally regarded as the probable centre of origin of a taxon (Cain, 1944; Good, 1953). Out of the 9 genera and c. 90 species which constitute Persoonieae, 5 genera and c. 77 species are found in Australia (and two more in the adjacent New Caledonia). As Good (1953) rightly points out and illustrates in the case of Asclepiadaceae species concentration alone should not be relied upon but the distribution of the primitives should also be taken into account in fixing the centre of origin of a taxon. Usually diploids, being more primitive and less adaptable than their polyploid derivatives, will have limited range and do not stray far from the place of their origin while their

polyploid derivatives have greater spread e.g., *Tradescantia* (Anderson, 1937). The two diploid genera of the Proteaceae (*Bellendena* and *Persoonia*) are confined to Australia. Australia, therefore, seems to be the centre of origin of the tribe Persoonieae.

Within Australia, all the 5 genera of the Persoonieae are found in East Australia including Tasmania, which seems to have been connected with the mainland in the past (Crocker and Wood, 1947; Browne, 1945), while only *Persoonia* is represented in West Australia. Even within this genus, there are more species in East (43) than in West (27). East Australia, therefore, seems to be the centre of origin of the tribe Persoonieae, wherefrom ancestral stocks probably migrated to the other land masses.

It is interesting to notice that in *Persoonia* as well as 11 other genera of the Proteaceae common between East and West Australia, there are no species common between the two zones. This leads one to the conclusion that there existed in the past a pan-Australian flora, as Diels (1906) also believed. After evolution has occurred to the generic level, ecological and geographical barriers seem to have been created between the two regions which led to independent speciation. Geological history shows that during Cretaceous, the central part of Australian continent was under sea so that the land presented the form of two long islands, eastern and western (Crocker and Wood, 1947).

All the 7 species of *Picnostylis* section of *Persoonia* are mainly confined to W. Australia, only *P. falcata* extending to N. Australia and Queensland. This section which contains the only species with zygomorphic flowers among Persoonieae, therefore, seems to have evolved in W. Australia. A detailed study of the cytology and distribution pattern of the Epacridaceae, another typically Australian family led Smith-White (1948) to a similar conclusion; he observes: "the genera that are endemic in W. Australia are usually specialised relatives of the eastern ones, and data do not suggest W. Australia as the source of the whole group".

Evolution within the tribe Persoonieae as visualised by the writer is represented in Fig. 272.

ACKNOWLEDGEMENTS

The writer wishes to express his thanks to the Authorities of the Colombo Plan for the award of a Senior Research Fellowship during the tenure of which most of these studies were made in the Department of Botany, University of Tasmania, Hobart, and to the Authorities of the Andhra University, Waltair, for deputing him. The writer is grateful to Prof. H. N. Barber, University of Tasmania, for the facilities and encouragement and to Dr. W. M. Curtis, for help during the progress of the work. His thanks are due to Mr. Max Gilbert, University of Tasmania, Mr. L. S. Smith, Queensland Herbarium, Brisbane, Mr. R. Carolin, University of Sydney, the Director of Melbourne Herbarium and the Inspector of Forests, Noumea, New Caledonia, for the materials they kindly supplied and to the Department of Photography, Tasmanian University, for his photographs. He is also thankful to Dr. A. C. Joshi, F.N.I., for kindly going through the manuscript.

POSTSCRIPT : When this paper was in press, the article of Haber (Phytomorphology, 1959 pp. 325-358) appeared. While there is general agreement between the present writer and Haber in the evolutionary trends in the inflorescence and flower, there is difference of opinion in the interpretation of the morphology of the nectary. Haber interpreted the nectary as corolla and therefore that the flower is dichlamydeous or monochlamydeous by reduction. In addition to the arguments presented in this paper, the writer wishes to add the following to substantiate his view that the proteaceous flower is fundamentally monochlamydeous.

In interpreting the 'glands, discs and scales' as corolla or modified form of it, Haber has not properly weighed the evidence of their position. Though in the polyphyllous condition the lobes of the nectary occur in the position of petals, in the gamophyllous state, the gland stands *inner to the tepal-stamen whorl*. The writer is doubtful whether the corolla can or does occur inner to an epiphyllous staminal whorl in any angiospermous family,

It is generally accepted that gamophylly and zygomorphy of the corolla are progressive and irreversible changes. It is difficult to imagine how a change can occur during the process of reduction "from a gamophyllous corolla to scale-like organs or to glands" (Haber, p. 355).

In the families in which strong zygomorphy of the corolla has been attained, tubular or polyphyllous conditions are not usually noticed. A great diversity is seen in the structure of the nectary in the Proteaceae, not only within the family but even within genera e. g., *Grevillea*. In *G. leucoptervis* the gland is annular; in *G. crythmifolia* it is more than semiannular with a small lobe at the back of the stipe; in the majority of species it is semiannular and strongly zygomorphic while in *synaphae* it is absent. Such a variety is not seen in the corolla within a single family but is noticed in the structure of the nectary e. g., Cruciferae. The writer therefore feels that the nectary is not homologous to the corolla but to the gland of a dichlamydeous flower.

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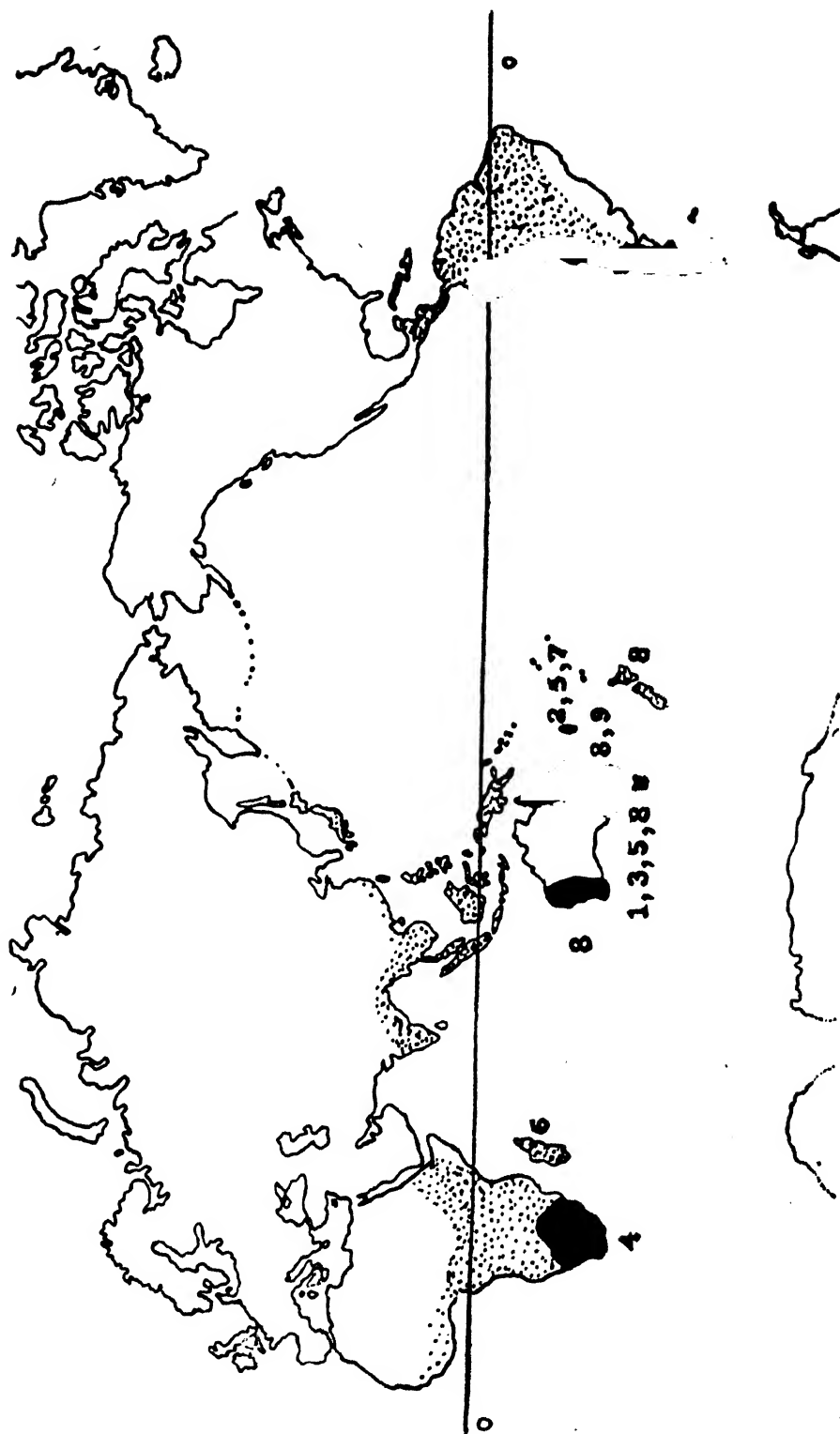
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- 1 *Bellendenkia montana* (Mount Wellington, Tasmania) in full bloom
- 2 A branch with inflorescences, inset is a branch with fruits
- 3 *Ceanothus velutina* (Florentine, Tasmania) in full bloom
- 4 A branch with inflorescences
- 5 A branch with fruits



7. A branch of *Pterospora alberti* (Flor. nino, Tasmania) with inflorescences.
 8. A branch of *Pterospora sancti* with flowers (W. Australia).
 9. A branch of *P. purpurea* (Tasmania) with flowers; inset is a branch with fruits.
 10. A branch of *P. portulaca* (New South Wales) with flowers; inset is a branch with fruits.
 11. A branch of *P. hirsutula* (N. S. W.) with flowers.
 12. A branch of *P. toa* (New Zealand) with flowers.



MAP 1.—Map of the world showing the present day distribution of the Proteaceae. Depth, of ink represents the density of species. Numbers indicate genera of Proteaceae : 1. *Agastichys*, 2. *Beauprea*, 3. *Bellendena*, 4. *Brabeium*, 5. *Cenarrhena*, 6. *Dilobea*, 7. *Garnieria*, 8. *Persoona*, 9. *Symphyonema*.

STUDIES ON INDIAN HYMENOPHYLLACEAE
PART I. CONTRIBUTIONS TO OUR KNOWLEDGE OF *CREPIDOMANES*
***LATEALATUM* (v.d.B.) COPELAND COMB. NOV.**

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(Communicated by S. N. Das Gupta, F.N.I.)

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ABSTRACT

The paper deals with the morphological and anatomical features of a Hymenophyllaceous fern, *Crepidomanes latealatum* (v.d.B.) Copeland comb. nov., collected from Mercara in Coorg (South India). A detailed account is given of the leaf, rhizome, sporangial initiation, spore formation and some cytological stages.

The rhizome is totally devoid of roots although slender branches of the rhizome look like roots and perhaps behave as such. The stem stele is a centrarch protostele. A trace from this is abstricted (without any gap) and splits at the petiolar base into a leaf trace and an axillary shoot trace. The leaf is highly lobed, has dichotomous venation, free veinlets and a lamina generally one cell thick, except round the vascular bundles. False veinlets are present in the leaf, which are not connected with the midrib, a characteristic feature of this species. Dermal appendages are unicellular hairs with saccate bases, found on the rhizome and on the petiole, wherever not covered by the lamina. The involucre is of the obconic type fused in the lower portion and bivalved above, valves being entire. The receptacle is cylindrical and slightly exserted. The sorus is gradate and basipetal in development. The sporangia are 320μ long, having narrow and elongated surface cells, with oblique and complete annulus of 20 to 22 cells, opening by a transverse slit marked by a couple of thin walled large cells. The development of the sporangium is of the leptosporangiate type and is initiated by a single cell. The spore mother cells are sixteen in number and the spores number 60-64. The spores are tetrahedral in form and measure 42μ by 46μ . Certain cytological stages in the development of the spore are also described.

INTRODUCTION

This family of beautiful, translucent ferns is distributed all over India, chiefly in moist and shaded places in the Himalayas, Khasya hills, Western Ghats of the Bombay, Madras, Kerala and Mysore States, and Central Provinces (Beddome, 1883).

The family comprises of two genera, *Hymenophyllum* Linn. and *Trichomanes* Smith, according to Beddome (1883), Bower (1908, 1926) and Holttum (1948, 1954). The classification is based purely on indusium characters—in *Hymenophyllum* the indusium being two lipped while in *Trichomanes* it is tubular with the mouth being truncate or slightly two lipped. There are three genera, *Loxosoma* Br., *Hymenophyllum* Linn. & *Trichomanes* Smith, according to Hooker and Baker (1868); four genera, *Trichomanes* L., *Cardiomanes* Br., *Serpyllopsis* v.d.B. and *Hymenophyllum* Smith, according to Christensen (1938). But Copeland (1938, 1947) has divided the family into as many as thirtythree genera taking into account a number of criteria apart from the characters of the sorus, indusium and spore output etc.

The number of species recognised for the family also varies according to different authors, reaching to a maximum of 650 (Copeland, *loc. cit.*).

Very little work has been done on Indian Hymenophyllaceae. The only paper on the subject is on the cytology of some Indian species of Hymenophyllaceae by Mehra and Gurdipsingh (1957). It is proposed to study all the Indian members

of this family in detail and the present paper is the first of the series discussing the morphological, anatomical and some cytological features of a South Indian species of *Crepidomanes*, *C. latealatum*.

The material that has been studied comes under the genus *Trichomanes* according to Beddome. If criteria like sporangial characters and spore output are taken into account a comparison with the genus *Trichomanes* of Bower can also be made. According to the classification of Copeland, however, a comparison is possible only with the genus *Crepidomanes*, although in a preliminary note the species was tentatively referred to *Amphipterum* (Sharma, 1960). The presence of false veinlets, which are unrelated to the midrib of the lamina, is a special feature which suggests comparison with the species, *Crepidomanes latealatum*.

The material was identified as *Trichomanes latealatum* (v.d.B.) Christ. (*Didymoglossum latealatum* van den Bosch) by the authorities of the Kew Botanic Gardens which, according to Copeland's recent classification, happens to be *Crepidomanes latealatum* (v.d.B.) Copeland comb. nov. thus confirming the author's identification.

MATERIAL AND METHODS

The material, which was obtained in 1958 by Dr. S. B. Kausik from Mercara in Coorg was first preserved in formal-acetic alcohol and then transferred to 90 per cent alcohol, was very kindly made over to the author.

Sections were cut 12 to 16 μ in thickness. The stains used singly and in combinations were haematoxylin, orange G, saffranin and fast green. Maceration in a solution of nitric acid and potassium chlorate was done to clear up the vascular tissues. Phloroglucin was also used to stain the lignified tissues (Foster, 1942).

GENERAL MORPHOLOGY

The plants are epiphytic varying in size from 3 to 6 cms. in height and are characterised by creeping, jet black, filiform, branched rhizomes (Fig. 1, Pl. XIX), nearly 1 mm. in thickness, covered all over by unicellular hairs. The axillary branches are present more or less in the axil of every leaf. The leaves are translucent, finely dissected, having free dichotomous venation. Each narrow segment or pinnule has a single veinlet (Fig. 2, Pl. XIX). Pseudoveins as reported by earlier workers are also present in the leaf (Fig. 3, Pl. XIX) in addition to the normal veins. These are not definite vascular tissue and are only modified thickened cells resembling the bundle sheath cells of the true veins. According to Prantl (1875) (see Bower 1926) and Smith (1955), "they can hardly be anything else than the vestigial remains of true veins no longer functional." No stomata are present on the leaf surface, the reason being the thin nature of the leaf. Here and there in the lamina are found some thick walled scattered cells, that stain red with phloroglucin and hydrochloric acid. The exact function of these lignified cells could not be clearly made out.

A characteristic feature is the complete absence of roots in the plant, which has been noted by earlier workers. This is perhaps an adaptation to its epiphytic and hygrophilous habit. Their function is probably performed by the leaf-less branches of the rhizome covered with hairs resembling those normally found on the axis and leaf of rooted species, acting as substitutes for true roots (Bower, 1926).

The dermal appendages are unicellular hairs with saccate bases. They are found on the rhizomes and on the petioles wherever the lamina is not prominent. No palaeae orramenta are present.

The sorus is marginal or terminal on the lowest pinnules of the pinnae in the upper half of a leaf. Sometimes it is found that the sorus is confined to the ventral pinnules also (Figs. 1 and 2, Pl. XIX).

The involucre is of the obconic type, fused in the lower portion but bilipped above, the lips having entire margins. False veinlets are also present on the involucre flaps. The receptacle is cylindrical and is slightly exserted (Fig. 2, Pl. XIX). The sorus is gradate and basipetal in development, a characteristic feature of the whole family (Bower, 1926).

ANATOMY

Rhizome—

Figure 1 shows a portion of the rhizome cut longitudinally. Saccate bases of unicellular hairs are clearly seen. These form the outermost layer of the rhizome and these unicellular hairs constitute the only dermal appendages. Figure 2 is a transverse section of a rhizome. The cortex can be differentiated into an outer (o.c.) thickwalled black coloured, sclerenchymatous zone and an inner (i.c.) zone made up of two or three layered thickwalled parenchyma. The endodermal layer, with the casparian strips in the radial walls of the cells, can be clearly made out. The pericycle is one layered. The stele is protostelic represented by a few tracheids, surrounded by the phloem, which is interrupted on one side—the ventral side. Such a stele, described as subcollateral, has been reported in *Hymenophyllum tunbridgense* and *Trichomanes trichoides* (Boodle, *loc. cit.*).

The finer branches of the rhizome present an appearance almost like roots but transverse sections of these branches show an undifferentiated cortex but a protostele of 1-4 tracheids surrounded on all sides by phloem. Therefore the author presumes them to be the finer branches of the rhizome. Probably these branches perform the function of roots as has been pointed out by Bower (1926).

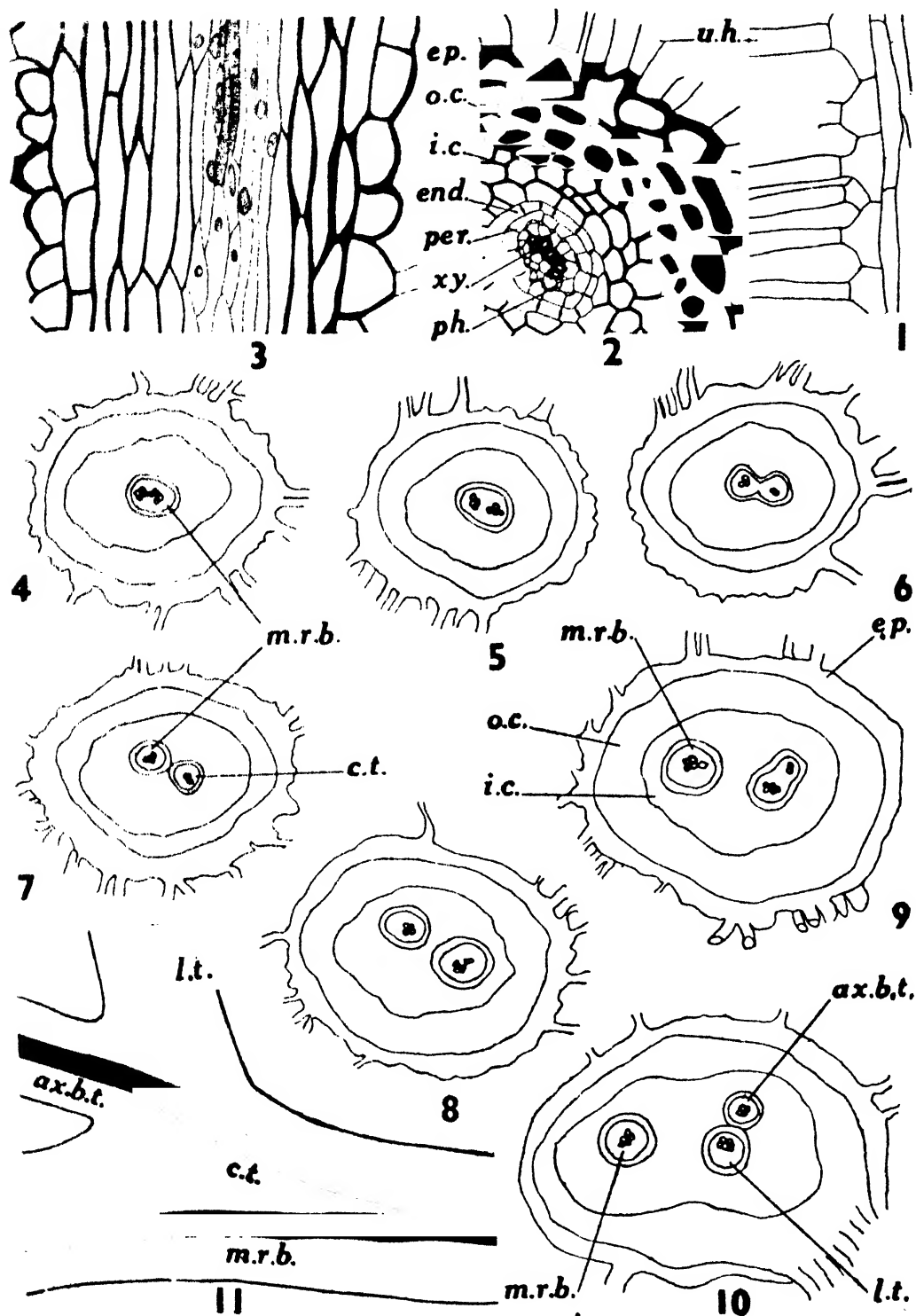
The stele, as judged by transverse sections of young and very old stems, shows a single mass of xylem tracheids of varying number—a definitely protostelic condition. The phloem, however, goes all round but not in a continuous ring. Often it may be less on one side, probably the ventral side of the rhizome than on the other; such a condition has often been described as an interrupted phloem (Boodle, *loc. cit.*).

Boodle has described a double band of xylem in *H. cruentum* enclosing a little parenchyma—a condition which can be interpreted as comparable to a siphonostele. Boodle has further pointed out that there is considerable variation in the stelar structure and that it could be correlated with the size of the plant. The author agrees with this view although her species does not show these variations. Here she finds invariably a single band of xylem.

In longitudinal section of the rhizome (Fig. 3) the sieve tubes are clearly seen with sieve plates. The xylem is formed of annular, spiral and scalariform tracheids.

Figures 4-10 are serial transverse sections of the rhizome and are diagrammatically represented. They show different stages of leaf trace sequence. A stele common to the leaf and axillary branch is first formed by the abstriction of the main stele (Figs. 4-7). After some distance this trace divides into two (Figs. 8 & 9) and forms the axillary branch trace and the leaf trace proper (Figs. 10 & 11). The pinna traces also come out in the same fashion by the abstriction of the leaf trace.

No leaf gaps are formed. The similarity between the main stele, the branch trace and the leaf trace has been noticed by earlier workers and has been interpreted as an evidence of the primitive position of the Hymenophyllaceae.



TEXT-FIGS. 1-11.

Leaf—

Figures 12-16 represent serial transverse sections of the leaf from below upwards. The lower part in each figure represents the dorsal side and not the ventral side of the leaf.

Figure 12 is a transverse section of the leaf in the basal portion. It is found that the petiole resembles the rhizome in all the details. The stele is concentric as in the rhizome but the phloem is more developed on one side and only one layer thick on the other side. The petiole is covered with unicellular hairs just like the rhizome. The cortex is divisible into an outer, very thick-walled sclerenchymatous zone and an inner less thick-walled parenchymatous cortex. The stele is encircled by a single layered endodermis and pericycle. Casparian strips on the radial walls of the endodermis can be clearly made out. A little higher up the stele becomes collateral (Fig. 13), which state is maintained till the apex of the leaf.

Fig. 4, Pl. XX shows a transverse section of the middle region of the leaf. The leaf is seen to be generally one cell thick except round the vascular bundles, where it is several cells thick. The larger bundle in the centre is the midrib and the smaller one is a lateral vein. No hairs are found at this level. At the base of the petiole there is a thick zone of sclerenchymatous cortex. This tissue gradually diminishes at this level and it finally disappears on the two lateral sides, from where the lamina comes out, but it persists above and below the midrib even in the apical region of the leaf (Fig. 14).

It is found that the xylem in the petiole always flattens out a little (Fig. 15 & Fig. 4, Pl. XX) before the pinna trace is formed (Fig. 16).

The structure of the leaf trace as seen in this species seems to support Boodle's opinion that species having a subcollateral stele in their rhizome, have a collateral structure in their petiole (Boodle, *loc. cit.*).

Sorus

The sorus is terminal on the lowest pinnules (Figs. 1 and 2, Pl. XIX). The receptacle is 2 mm. long, generally exserted, sometimes included and elongates by intercalary growth. The cup-shaped involucre is united at the base and bilipped higher up (Fig. 17 and Fig. 2, Pl. XIX). This partly fused condition has presented a lot of difficulty in the correct identification of the genus.

Figs. 18-21 are slightly oblique serial transverse sections of a young sorus from base to apex, showing the gradate development of sorus and some other points of interest (see also Fig. 5, Pl. XX). The lower portion of the involucre is three to four cells in thickness (Figs. 18 & 19) while the upper portion is only one cell thick (Figs. 20-21). Fig. 18 shows the receptacle (*r*) in the centre. No sporangia

EXPLANATION OF TEXT-FIGURES 1-11

Lettering—*ax.b.t.*, axillary branch trace; *c.t.*, common trace; *end.*, endodermis; *ep.*, epidermis; *i.c.*, inner cortex; *lt.*, leaf trace; *m.r.b.*, main rhizome bundle; *o.c.*, outer cortex; *per.*, pericycle; *ph.*, phloem; *u.h.*, unicellular hairs; *xy.*, xylem.

Fig. 1. Part of the outer layer of the rhizome showing the unicellular hairs, covering it. $\times 185$,

Fig. 2. Transverse section of the rhizome. $\times 185$.

Fig. 3. Longitudinal section of the rhizome. $\times 185$.

Figs. 4-10. Serial transverse sections of the rhizome showing the leaf and axillary branch trace sequence. $\times 75$.

Fig. 11. A macerated preparation of the rhizome showing the origin from the main trace of a common trace, which later divides into leaf and axillary branch traces, at the petiolar base. $\times 34$.

are borne at this level. Fig. 19 is the next higher section showing sporangia (*sp.*) in different developmental stages. Fig. 20 shows a section at a still higher level with the mature sporangia having a few spores in them. In Fig. 21 are represented only two separate single layered flaps of involucre. This being a young sorus the flaps extend beyond the tip of the receptacle but in older sori the receptacle proceeds beyond the flaps and becomes exerted.

The receptacular supply is a continuation of a laminar vein. In the material examined by the author, the vascular supply of the fertile pinnule splits up into three strands on entering the receptacle. The central one supplies the receptacle itself and the other two lateral ones enter the two valves along the line of their fusion (Figs. 18-20). The receptacular supply is more prominent in the basal portion but becomes less defined higher up, probably due to want of lignification.

The individual cells of the involucre contain many chloroplasts lining the cell wall (Fig. 22).

SPORANGIAL DEVELOPMENT

The sporangium measures 320μ long and is initiated by a single cell (Fig. 23), which is a big superficial cell of the receptacle. It is densely cytoplasmic and has a big nucleus. This cell divides transversely (Fig. 24) to form a lower stalk cell and an upper sporangial cell. The stalk cell divides longitudinally once or twice but does not develop any further. Nor do any of these cells elongate so that the adult sporangium remains almost sessile. The stalk cell merges with the receptacular cells and the exceedingly short stalk of the sporangium is only two cells thick (*st.*) fig. 27. The upper cell forms the sporangium proper, as in other leptosporangiate ferns (Eames, 1936), by further divisions. The first division of this cell is oblique, dividing the sporangial cell into two unequal halves (Fig. 25). The bigger cell divides obliquely again resulting in the formation of three cells (Fig. 26). By the periclinal division of the upper cell a central triangular cell is outlined (Fig. 27). The peripheral cells start dividing (Figs. 28-37) and ultimately form the wall. Meanwhile the central triangular cell undergoes a few divisions with walls at right angles to each other (Figs. 29 & 30). Periclinal divisions also occur again and outline a central mass of cells now separated from the sporangial wall by two layers (Figs. 35-37). These become the tapetum while the central mass of cells repeatedly divide to form the spore mother cell (Figs. 34-37). Each spore mother cell has got a single nucleus with 4 or 5 nucleoli and dense cytoplasm. Macerations show that each sporangium shows 16 spore mother cells. These divide by subsequent divisions to form 64 spores.

EXPLANATION OF TEXT-FIGURES 12-22

Lettering—*chl.*, chloroplast; *ep.*, epidermis; *end.*, endodermis; *in.*, involucre; *i.c.*, inner cortex; *o.c.*, outer cortex; *p.*, pinna; *p.t.*, pinna trace; *per.*, pericycle; *ph.*, phloem; *r.*, receptacle; *sp.*, *sp.*, sporangium; *xy.*, xylem.

Fig. 12. Transverse section of part of leaf in the basal region. $\times 175$.

Fig. 13. Transverse section of the leaf passing through its middle showing only xylem and phloem. $\times 410$.

Fig. 14. Transverse section of the leaf from its apex. Note the reduction in sclerenchyma. $\times 170$.

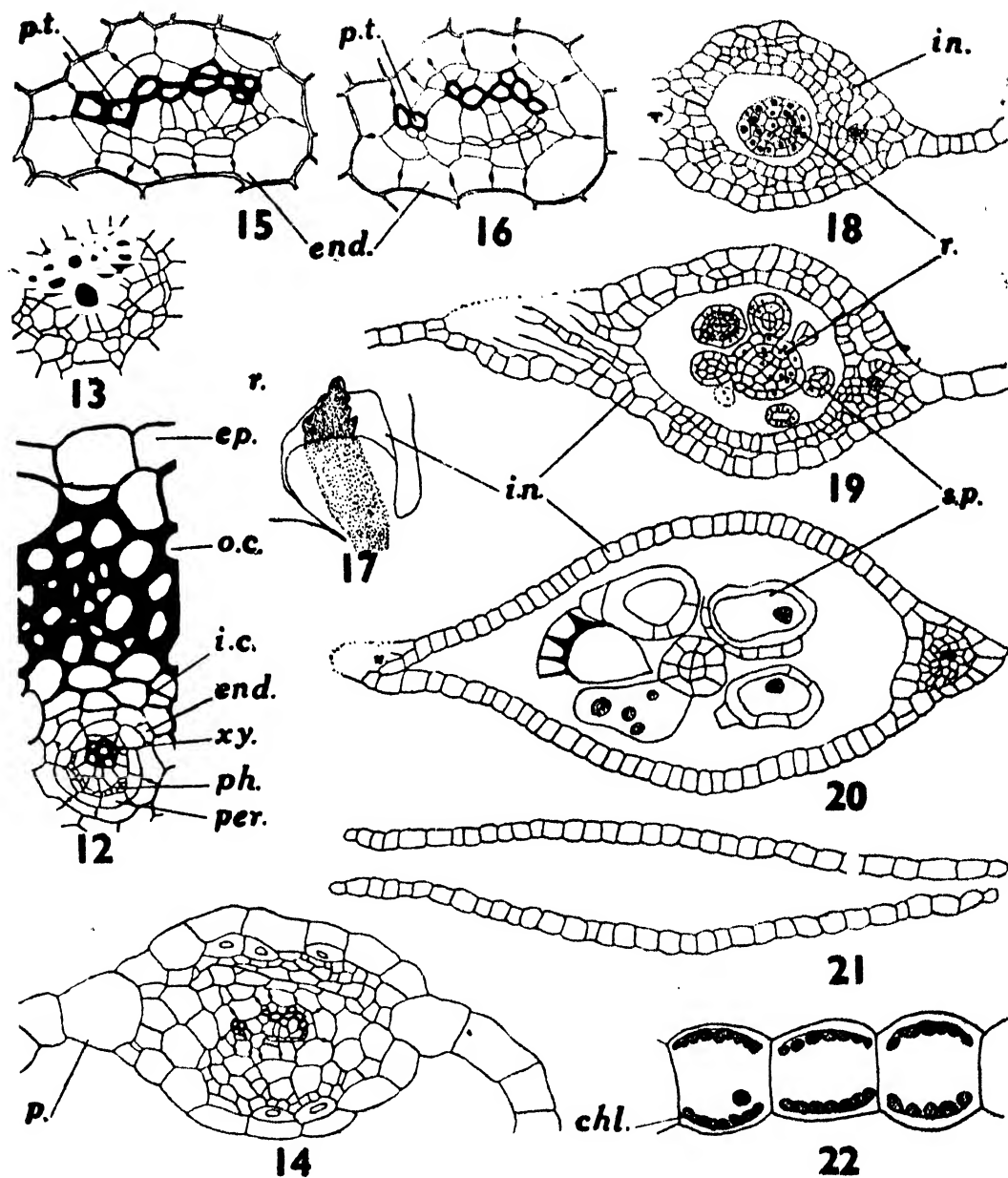
Fig. 15. Only the stele is magnified to show the tissues. The xylem is in the form of a plate and is ready to give a pinna trace. $\times 430$.

Fig. 16. The pinna trace has separated out from the main bundle. $\times 375$.

Fig. 17. The bilipped involucre showing the partly exerted receptacle in the centre. $\times 10$.

Figs. 18-21. Transverse section of the receptacle at different levels from base to apex (explanation in text). $\times 80$.

Fig. 22. Cells of the indusium showing the chloroplast. $\times 260$.



TEXT-FIGS. 12-22.

A single row of cells, from the sporangia wall, forms the annulus (Fig. 38). The wall cells are elongated. The annulus is made up of 20 to 22 cells and oblique, opens by a transverse slit marked by a couple of thinwalled large cells (Fig. 38). The sporangia are placed horizontally on the receptacle (Fig. 5, Pl. XX). The annulus is directed towards its receptacular apex. A lateral view of the sporangium (Fig. 38) shows that the inner wall of the annulus and its radial walls are also very thick. The outer wall is comparatively thin. A surface view of the sporangium (Fig. 39) shows that the annulus is broad.

CYTOLOGY

All the cytological stages, leading from the spore mother cells to the spore formation stage, are seen. The spore mother cells are pyriform structures with big nuclei, having four or five nucleoli, a faintly staining reticulum and dense cytoplasm (Figs. 37 and 40).

First Division of Meiosis (Heterotypic divisions).

Prophase -

Fig. 40 is a section of a young sporangium showing two annulus cells (*an*) on either side, the two layered tapetum and the central mass of spore mother cells. The inner layer of tapetum can be seen to be disorganising (Fig. 40, see also Figs. 41 & 43-45). The spore mother cells are pear-shaped with their narrow ends facing inwards. The nucleus is situated at the periphery, with thread like, darkly staining reticula seen in different stages of development and cut at various levels (Fig. 40).

Fig. 41 is a late prophase stage. The chromosomes are now organised into small bodies. They may be of varied shapes but are always short and sometimes seen to be divided or forked (Fig 42 & Figs. 6, Pl. XX). The chromosomes resemble those of *Trichomanes plicatum* v.d.B. (*Crepidomanes plicatum* v.d.B. Copel.) as studied by Mehra and Singh (1957, Pl. 16, photo 15). The number of chromosomes could not be counted because of the lack of fresh material.

Metaphase -

Fig. 43 shows the metaphase stage of the first meiotic division. At this stage the spore mother cells lie irregularly in the sporangial cavity. The spindles are formed and the chromosomes arrange themselves at the equatorial plane (Fig. 43 & Fig. 7, Pl. XX). The inner layer of the tapetum is more or less completely disorganised.

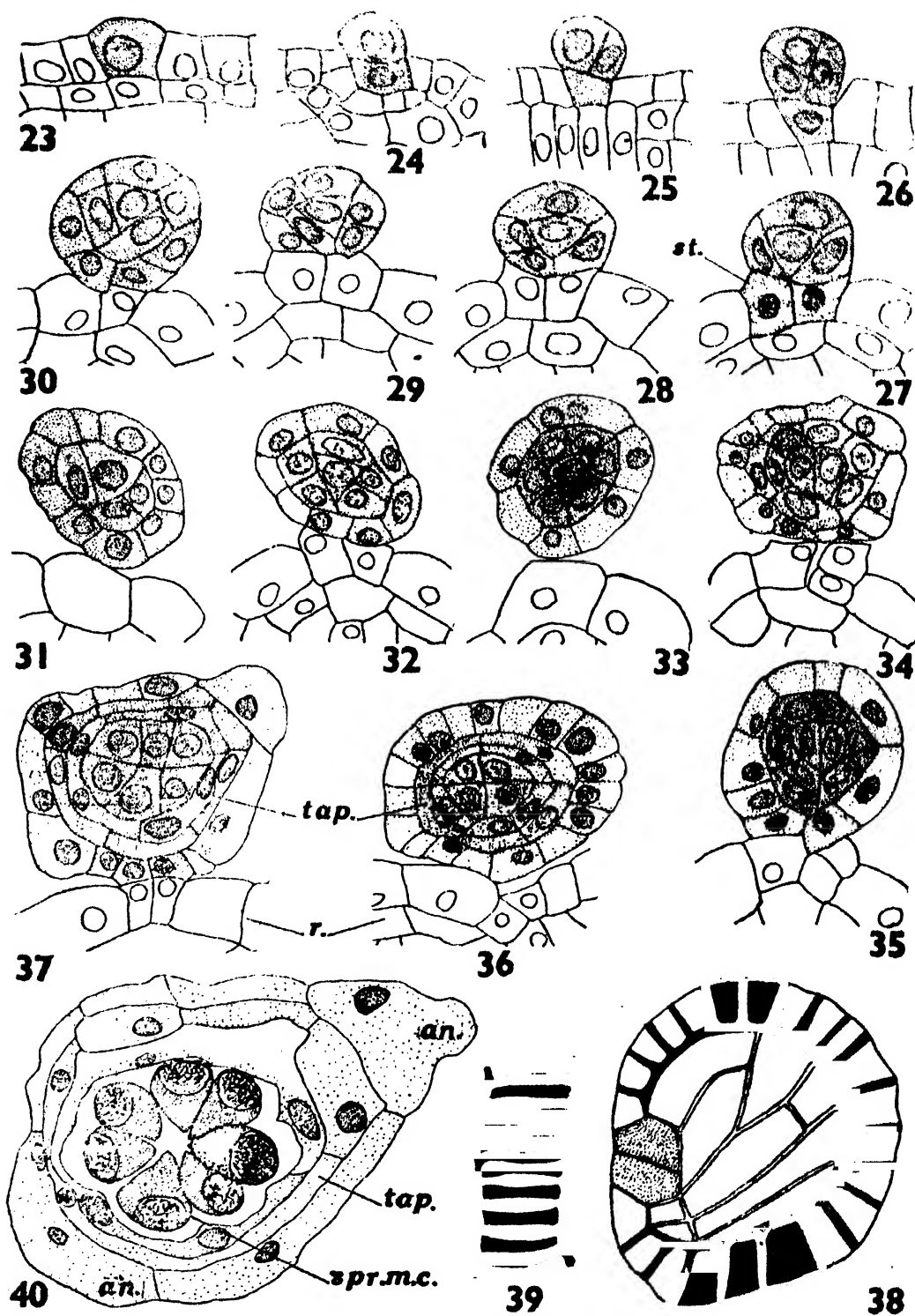
EXPLANATION OF TEXT-FIGURES 23-40

Lettering—*an*, annulus; *r.*, receptacle; *spr.m.c.*, spore mother cell; *st.*, stalk; *tap.*, tapetum. Figs. 23-37. Stages in the sporangium formation from a single cell to a fully developed sporangium. For explanation see the text. $\times 375$.

Fig. 38. A fully developed sporangium showing the annulus and elongated, narrow cells of the sporangium wall. $\times 200$.

Fig. 39. The annulus cells seen in surface view. $\times 260$.

Fig. 40. The sporangium showing the early prophase stage. The thread like, darkly staining reticula are seen in different stages of the development and are cut at different levels. $\times 375$.



TEXT-FIGS. 23-40.

Anaphase & Telophase—

A possibly late anaphase or early telophase stage is shown in Fig. 44. The chromosomes have collected at the poles as can be made out in the polar or equatorial views of the spindles in the spore mother cells.

Fig. 45 shows the late telophasic stage with the cell plate and the nuclei at the two poles. The spindles are disappearing gradually. The inner tapetal layer is completely disorganised.

Fig. 46, 47 and Fig. 8, Pl. XX are the bilateral and tetrahedral spindles of the second division. Such divisions are not very frequent. The author presumes that this is due to a very short interphasic duration. It is also clear from the above figures that the spindles can be tetrahedral as well as bilateral and that the cell plate of the first division persists for some time.

Fig. 48 shows three nuclei out of four belonging to a tetrad. Examinations of sections showed that all these four nuclei are connected by spindles of cytokinesis so that there are actually six spindles connecting four nuclei. This has been just figured in *Isoetes coromandelina* (Ekambaranathan & Venkatanathan, 1933) (Fig. 39). There are two possible explanations for Fig. 48. One is that the two spindles of the earlier divisions persist and four are formed a new. A second explanation is that extra spindles are formed afresh. Sharp also records the occurrence of such extra spindles in *Nicotiana* (1934). The author presumes that the same explanation holds good in *Crepidomanes latealatum*. The cell plates are laid along the equatorial plane of these cytokinetic spindles. In the ripe sporangium, tetrads with these cell plates can be clearly seen (Fig. 49). The next stage noticed is rather advanced and shows the formation of four spores whose tetrahedral arrangement can be easily seen (Fig. 50). In all 64 spores are produced in each sporangium, all of which seem to be viable.

EXPLANATION OF TEXT-FIGURES 41-52

Lettering— an., annulus; c.pl., cell plate; chr., chromosomes; nuc., nucleus; r., receptacle;

spr.m.c., spore mother cell; spr.t., spore tetrad; t.r.m., tri-radiate mark; tap., tapetum.

Fig. 41. A late prophase stage showing the short and thick chromosomes. $\times 375$.

Fig. 42. Only one spore mother cell magnified to show the short and thick chromosomes. $\times 850$.

Fig. 43. The heterotypic metaphase stage. The inner layer of tapetum starts disorganising. $\times 375$.

Fig. 44. A late anaphase or early telophase stage. Polar or equatorial views of the chromosomes and the spindles are seen. $\times 375$.

Fig. 45. A late telophase stage. A thick cell plate is seen at the equatorial plane of each spindle. $\times 375$.

Fig. 46. A spore mother cell showing a bilateral homotypic metaphase stage. $\times 700$.

Fig. 47. Another spore mother cell with tetrahedral spindle. $\times 700$.

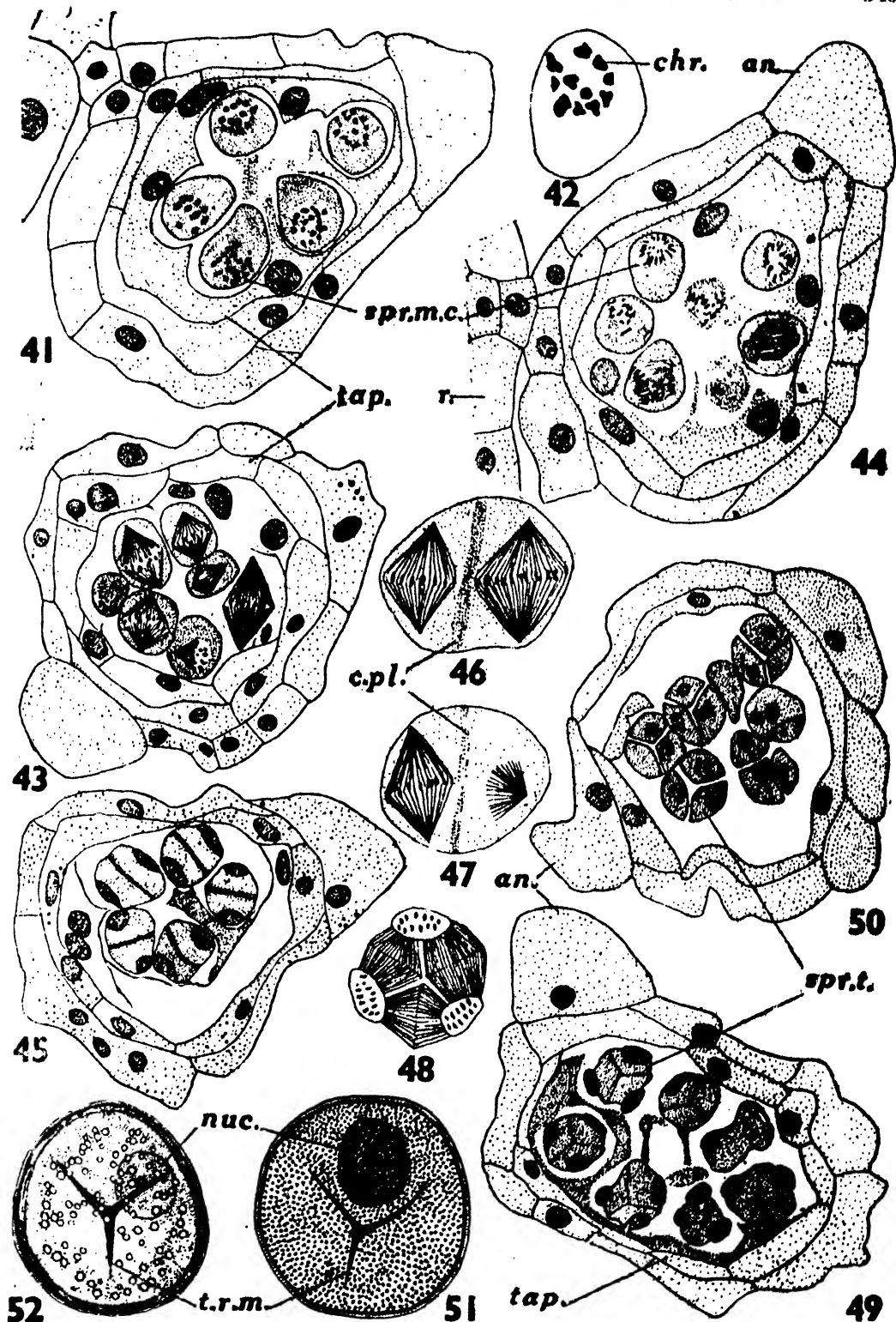
Fig. 48. Spore mother cell showing three nuclei connected with 3 sets of cytokinetic spindles. The cell plates are not yet united. Other three sets of the spindles could be seen on the other side connecting these three nuclei with the fourth one. $\times 700$.

Fig. 49. The sporangium showing spore tetrads not yet separated. The spindles have disappeared and the incomplete cell plates of the 6 sets of spindles have united in the centre. $\times 375$.

Fig. 50. Tetrad of spores in the sporangium. $\times 375$.

Fig. 51. A mature spore in surface view. $\times 850$.

Fig. 52. Section of the spore showing the different layers of the wall, the nucleus and the tri-radiate mark. $\times 700$.



TEXT-FIGS. 41-52.

SPORES

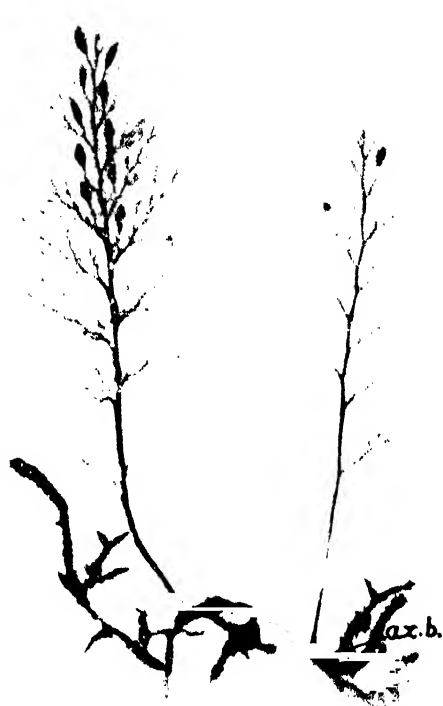
The spores are homosporous, globose in structure, with the triradiate mark on the proximal side (Fig. 51) and measure 42μ by 46μ in diameter. The thin outermost layer of the sporewall is smooth. Next to this comes the spiny layer. The innermost layer is thick and stratified (Fig. 52). Fig. 52 is a section of the spore and it shows the wall, nucleus, oil globules, cytoplasm and triradiate mark. The spore contents include some starch grains also. In the peripheral portion of the spore the cytoplasm is of a slightly denser consistency than the central part (Fig. 52).

ACKNOWLEDGEMENTS

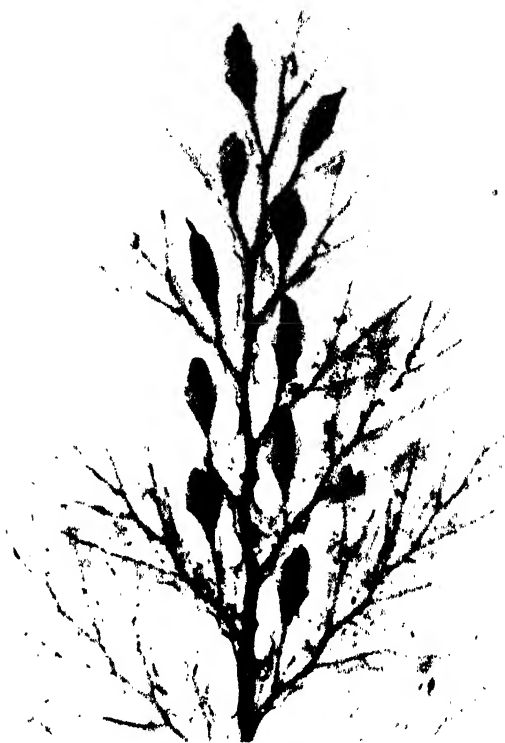
The author is highly grateful to Dr. A. R. Rao for his kind and valuable guidance during the course of the present investigations. She is indebted to the authorities of the Kew Botanic Gardens for the identification of the material, to Dr. S. B. Kaushik for kindly placing the present material at her disposal and to Mr. S. K. Nath for taking the photomicrographs. The author is also deeply indebted to Prof. S. N. Das Gupta F.N.I., for the interest he has evinced in her work.

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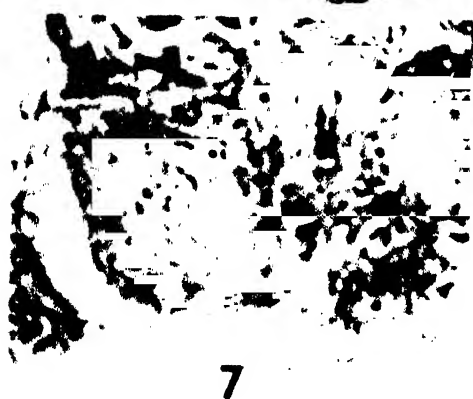
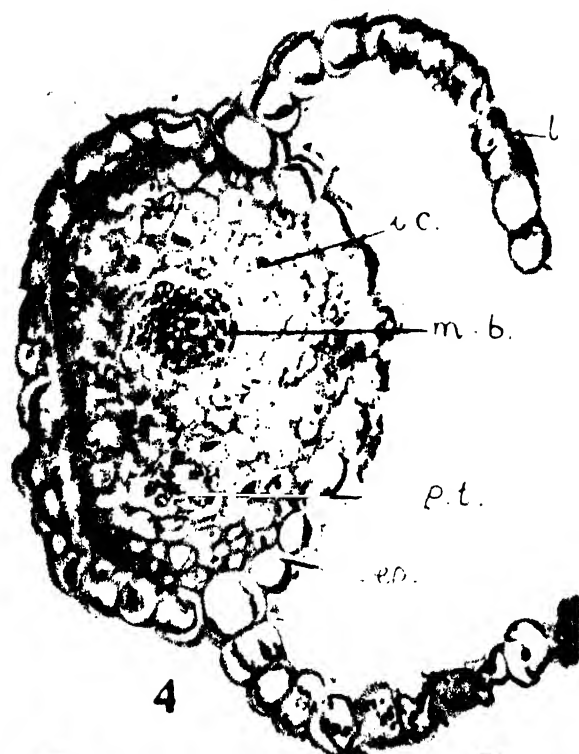
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EXPLANATION OF PLATES

All photographs are from untouched negatives.

PLATE XIX

- Fig. 1. Part of plant showing two leaves with receptacles and the filiform jet black rhizome with axillary branches. (*ax.br.*). $\times 1.4$.
 Fig. 2. Apical part of leaf showing the bilipped indusia and slightly exserted receptacle bearing sessile sporangia all round. The dichotomous venation of the pinnules are clearly seen $\times 4$.
 Fig. 3. A small portion of the leaf lamina magnified to show the false veinlets unconnected with the dichotomising midrib. $\times 35$.

PLATE XX

- Fig. 4. Transverse section of the middle region of the leaf showing the midrib bundle *an*; the pinna trace. (*ep.*, epidermis; *i.c.*, inner cortex; *l.*, lamina; *m.b.*, midrib bundled *o.c.*, outer cortex; *p.t.*, pinna trace). $\times 160$.
 Fig. 5. Longitudinal section of the receptacle showing the sporangia on it in different stages of development. The involucre can be seen to be 3 cells thick in the fused cup-like basal part and single layered higher up. (*in.*, involucre; *r.*, receptacle; *sp.*, sporangium; *an.*, annulus.) $\times 102$.
 Fig. 6. Three spore mother cells with short and thick chromosomes. The nuclear wall disappears at this stage. $\times 1045$.
 Fig. 7. The heterotypic metaphase stage showing the bipolar spindle. The chromosomes are arranged at the equatorial plane. $\times 1179$.
 Fig. 8. A homotypic metaphase stage showing two spindles lying side by side and the chromosomes arranged in the equatorial plane. The two spindles are separated from each other by a thick cell plate formed in the first heterotypic division. (*c.pl.*, cell plate.) $\times 1179$.

ALL TEXT-FIGURES are *camera lucida* sketches. In figs. 40-50 the forms of the chromosomes are slightly diagrammatically represented as they are not clearly visible.

COMPARATIVE AND FUNCTIONAL MORPHOLOGY IN THE MONO- GENEAN HAPTOR AS REVEALED IN THE MOST ADVANCED TYPES*

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ABSTRACT

Direct observations are made on two juvenile specimens one belonging to *Lithidiocotyle secunda*, the other belonging to *Kannaphallus univaginalis*, one adult *Heteraxine indica* and a long series of post-oncomiracidia, juveniles and immature *Pricea* spp. which afforded evidences, presented for the first time, with regard to the origin and homology of the functional haptor in the superfamily Dielidophoroidea of Price (1936). The evidences suggest that the superfamily is a heterogeneous assemblage of two distinct groups; one group with four clamps on each side and the functional haptor being formed from the anterior two-thirds of the larval haptor and the other group with the potentiality of unlimited addition of clamp units in metameric succession in which the functional haptor includes the anterior two-thirds of the haptor, though by far the greatest part is a newly formed entity in direct continuation forwards of the larval haptor. This study justifies the separation of the families Microcotylidae, Gastrocotylidae and Axinidae into a superfamily Microcotyloidea, effected by Unnithan (1957) without assigning any valid reasons.

The changes that occur in the post-oncomiracidia of *Pricea* spp. to the juveniles are described for the first time and comparisons are made with the developmental stages described for *Gastrocotyle trachuri* and *Pseudaxine trachuri* by Llewellyn (1959).

INTRODUCTION

The taxonomic importance of the functional haptor in the superfamily Dielidophoroidea Price (1936) cannot be over-emphasized. The origin and homology of the functional haptor of this group as to whether, it is a newly formed structure anterior to the larval haptor or whether it is formed from the larval haptor itself has remained ambiguous hitherto. Sproston (1946) holds the former view whether Frankland (1955) and Llewellyn (1957) hold the latter view. Neither of these views is adequately substantiated in their publications.

MATERIAL

The material studied included two juvenile specimens one belonging to *Lithidiocotyle secunda* Tripathi (1954) and the other belonging to *Kannaphallus univaginalis* Ramalingam (In press); one adult specimen belonging to *Heteraxine indica* Ramalingam (in press) and a long series of post-oncomiracidial larva, juvenile and immature *Pricea* spp. obtained from the gills of *Scomberomorus guttatus*, *Caranx sexfasciatus* and *Scomberomorus guttatus* respectively.

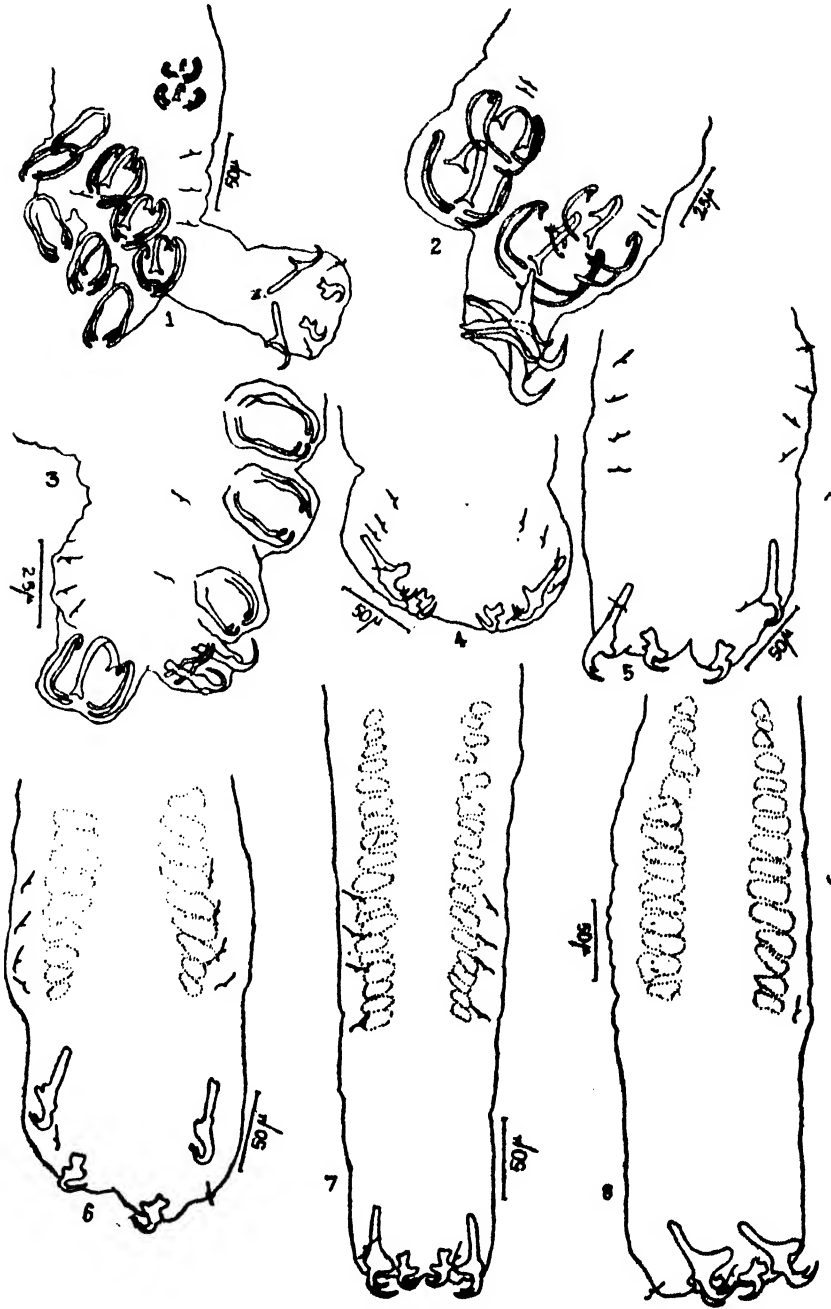
DESCRIPTION

While examining a number of specimens of *Lithidiocotyle secunda* from the smallest having 3/3 clamps to the largest adult having 133/136 clamps, it was

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observed that a single juvenile having 6/4 clamps on the haptor retained, besides the two pairs of anchors, six marginal hooklets (Fig. 1). Of these six hooklets,



TEXT-FIGS. 1-8.

- Fig. 1. Haptoral region of the juvenile *Lithidiocotyle secunda* showing the marginal hooklets.
 Fig. 2. Haptoral region of the juvenile *Kannaphallus univaginalis* showing the marginal hooklets.
 Fig. 3. Haptoral region of the adult *Heteraxine indica* showing the marginal hooklets.
 Fig. 4-8. Showing the metamorphic changes that take place in the haptor of the post-oncomiracidial larva of *Pricea* spp. during growth.

two are present in between the two dissimilar anchors, one on each side. Of the remaining four hooklets three are on the haptor by the side of the three posterior-most clamps and about the same level, whereas the fourth one is on the opposite side of the haptor near the level of the third clamp from the posterior end. The peduncle of the clamps arise inner to the hooklets.

In a collection of 269 specimens belonging to *Kannaphallus univaginalis* (representing from the juvenile with 1/1 clamp to the largest adult with 26 clamps on the longer side of the haptor) a single specimen having 2/2 clamps retained four marginal hooklets (Fig. 2) besides the two pairs of anchors. These hooklets are in front of the proximal two clamps, two on each side.

Of the four adult specimens belonging to *Heteraxine indica* examined, one retained five marginal hooklets (Fig. 3, Pl. XXI, Fig. 1). It has 34 clamps on its left side and one clamp on the distal end of the right side of the haptor. Of the five hooklets, three are present in front of the solitary distal clamp and the other two hooklets are present on the left side by the side of the two posterior-most clamps at about the same level.

The earliest post-oncomiracidial larva of *Pricen* spp. (Pl. XXI, Fig. 2) agrees with the larva of *Gastrocotyle trachuri* described by Llewellyn (1959) in the general characters, viz., in the absence of cilia, eye spot, in the presence of buccal suckers and the oesophagus being bifurcated but differs from the latter in the presence of marginal hooklets. May be, the present form recorded might represent an earlier stage to that described by Llewellyn. The following description deals with the changes that take place during the metamorphic growth of the post-oncomiracidial larva of *Pricen* spp.

Figures 4 to 8 represent the changes that occur in the haptoral region during the metamorphic growth of the post-oncomiracidial larva of *Pricen* spp. to the juvenile stage.

The haptor is discoid and has undergone no modification in the earliest post-oncomiracidial larva (Fig. 4, Pl. XXI, Fig. 3): it is 0.09 mm. long and 0.15 mm. broad in a larva measuring 0.50 mm. long and 0.15 mm. broad. With the growth of the larva, the posterior region of the worm elongates (Fig. 5, Pl. XXI, Fig. 4) to form a distinct median terminal lappet and the two pairs of anchors as well as the posterior pair of marginal hooklets between them are pushed backwards. This happens in a 1.34×0.13 mm. larva. A similar observation was made in the larva of *Pseudaxine trachuri* by Llewellyn (1959) wherein the anchor bearing lappet elongates considerably with a difference viz., the surviving oncomiracidial posterior hooks and post-oncomiracidial hooks are relatively further apart but in the present case the expansion occurs in the region between the lateral hooklets and posterior hooks, the post-oncomiracidial hook lying in the region of the posterior hooklet is also carried with it. The lateral wings flank the posterior region of the body for a length of 0.08 mm. and it gives the appearance of a flower vase. It has on them four pairs of marginal hooklets. The terminal lappet is 0.13 mm. long.

The first pair of clamp anlagen is seen at the level of the fourth pair of marginal hooklets (from the proximal end of the haptor), thence successively forwards. Clamp anlagen arises just medially to the hooklets. A similar observation is made on the juvenile *L. secunda*. In the 1.43×0.19 mm. post-oncomiracidial larva (Fig. 6, Pl. XXI, Fig. 5), the length of the haptor from the anteriormost clamp anlagen to the tip of the terminal lappet is 0.27 mm. the lateral wings extend for a length of 0.08 mm. and the terminal lappet is 0.15 mm. long.

Successive clamp anlagen are seen in the proximal region of the haptor as the post-oncomiracidial larva grows. In the 1.60×0.21 mm. post-oncomiracidial larva (Fig. 7) the length of the haptor from the anteriormost clamp anlagen to the tip of the terminal lappet is 0.40 mm., the lateral wings extend for a length of 0.07 mm. and the terminal lappet is 0.17 mm. long.

Thus while the post-oncomiracidial larva grows as a whole, the region bearing the hooklets does not show any apparent growth; the terminal lappet itself grown only to a maximum of 0.17 mm. in 1.80 mm. larva (Fig. 8). The growth of the terminal lappet does not seem to proceed further and in the adult measuring 6.67 mm. and having 110 clamps, is only 0.17 mm. long and does not grow further. The lateral marginal hooklet present on one side of the haptor of the larva is the fourth hooklet.

Clamp anlagen are seen subsequent to the elongation of the haptoral region into the two distinct regions (Fig. 5): the anterior region consisting of the lateral wings that flank the posterior region of the body and the posterior region consisting of the median terminal lappet.

Gallien (1934) thought that the adult clamps of *Diclidophora luscae* were formed round the lateral hooklets of the larvae. Frankland (1955) said that the clamps of *Diclidophora denticulata* are formed at the level corresponding to the larval hooklets and external to them. Neither of these authors have adequately substantiated their contention with figures. Frankland also states that in the development of *Diplozoon paradoxum* studied by Zeller (1872) the adult clamps are formed at the level of the corresponding pair of larval hooklets and this is not substantiated with figures. The present observation agrees with the views expressed by Gallien and Frankland but differs in that the clamps are formed internal to the hooklets in the post-oncomiracidial larva.

The four pairs of marginal hooklets are present in the haptor of the post-oncomiracidial stage as it retains the discoid form of the haptor of the oncomiracidium. With the elongation of the posterior region of the haptor the hooklets are lost in succession from forwards and this is inferred from the observation on the post-oncomiracidial larva retaining the distal-most two pairs of hooklets in the lateral wing like expansion of the haptor. Of the 34 specimens of the post-oncomiracidial larvae examined, six retained all the four pairs of hooklets, five retained a maximum of four hooklets (posterior two pairs), while the remaining 23 have lost all the lateral hooklets. These hooklets are lost much earlier to the differentiation of the functional clamps, even though, in the majority of the larvae the clamp anlagen are seen. This seems to suggest that the hooklets play only a subsidiary part in attachment. But Frankland (1955) is of the opinion that in *Diclidophora denticulata* the lateral hooklets disintegrate by the time clamps at the level of the corresponding hooklets take over their (hooklets') function.

The fifth pair of marginal hooklets between the two dissimilar pair of anchors are retained in the worm having in all 22 clamps.

Thus direct observations have now been made on the post-oncomiracidial larvae and juveniles belonging to three genera namely *Pricea*, *Lithidiocotyle* and *Kannaphallus* and besides on an adult worm belonging to the genus *Heteraxine* which provide basic evidence for the site of origin and homology of the adult haptor in relation to the haptor of the oncomiracidium larva. For the first time figures are now given showing the relative position *in situ* of the larval hooklets which were drawn from the specimens in my collection. They are deposited in the collections of the Zoological Survey of India, Calcutta.

It is observed that the functional haptor in the subfamily Priceinae (Gastroscoptylidae) in which the metameric succession of clamp units is unlimited include the anterior two-thirds of the larval haptor, though by far the greatest part of the adult haptor is a newly formed entity in direct continuation forwards of the larval haptor. There is a strong presumptive evidence that at least in *Diclidophora* spp. the functional haptor, in which the number of clamps is limited to four on each side, is formed entirely by the anterior two-thirds of the larval haptor.

DISCUSSION

Bemley (1942) found that in *Microcotyle spinicirrus* the clamps are formed anterior to the level of the larval haptor. From the preliminary study of Sandars (1944) on *Diplosiorcotyle johnstoni*, Frankland (1955) infers that the successive clamps may be added both before and behind the first formed clamps. Sproston (1946) in her diagnosis of the superfamily Dielidophoroidea includes the statement that the adult haptor is formed anterior to the level of the larval haptor in all families: a point which in view of the subsequent discoveries call for emendation (see below).

These observations lead to a further inference. In the Suborder Monopisthocotylea, and the superfamily Polystomatoidea of the Suborder Polyopisthocotylea, the larval haptor is transformed as a whole into the adult haptor. In the four examples studied in this work, belonging to the families Axinidae and Gastrocotylidae, only the anterior two-thirds of the larval haptor contributes to the adult haptor.

In the members of the families Microcotylidae, Axinidae and Gastrocotylidae the adult haptor has an entirely novel character in that its metameric succession of units (clamps) is not restricted to four on each side of the haptor as is the case in the rest of the families listed in the superfamily Dielidophoroidea.

Intrinsically, members of the family Gastrocotylidae like Microcotylidae and Axinidae have an indefinite capacity for growth, and this is almost uninhibited in the members of the subfamily Pricinae. The addition of clamps in the members of this subfamily is much more extensive than in Gastrocotylinae: a total of 140 clamps are present in a specimen of *Pricca* sp. and a total of 269 clamps in a specimen of *Lithidiocotyle secunda* were observed. On the other hand, in Gastrocotylinae the addition of clamps is much less extensive: a maximum of 30 clamps is recorded in *Gastrocotyle* and 37 clamps in *Pseudaxine*.

Moreover, clamps in Pricinae show great variations in their structure, as seen in *Lithidiocotyle* and *Pricca*, even though the fundamental plan in both is gastrocotylid. Whereas in the members of the subfamily Gastrocotylinae, the clamps have not undergone any modifications and they are all of the primitive type.

In the post-oncomiracidial larva of *Pricca* spp. two to three clamp anlagen are formed (Figs. 7 and 8) in the zone between the successive marginal hooklets; whereas in *L. secunda* and *Heteraxine indica* a single clamp only is observed against each hooklet (Figs. 1 and 3). It is probable that in *Pricca* spp. the clamps are formed at a more rapid rate to begin with than in *L. secunda*. During the later period of growth *L. secunda* acquires a larger number of clamps than do *Pricca* spp. Thus a specimen of *L. secunda* measuring 6.45 mm. has in all 269 clamps, while a specimen of *Pricca* sp. measuring 5.80 mm. has in all 140 clamps both of which are in the author's collection.

To summarize, the adult haptor in Heteraxininae (Fam: Axinidae) and Pricinae (Fam: Gastrocotylidae) includes the anterior two-thirds of the larval haptor, and contributes to by far the greater part of the newly formed entity, and it is in direct continuation forwards of the larval haptor. In the Dielidophoroidea, the addition of clamps is restricted to four on each side. And in fact, from the ontogenetic evidence brought forward here, the anterior limit of the adult haptor is precisely identical to that of the larval haptor in morphology. In this respect it presents a sharp and significant contrast to the members of the families Axinidae and Gastrocotylidae.

A convincing echo of phylogeny in ontogeny is seen in Fig. 1, where the first four clamp-pairs on the larval haptor in *Lithidiocotyle secunda* are in a double

median row. These are of a somewhat different shape to the first formed additional clamps of the adult haptor which have begun to form in front of the margin of the elongated larval haptor.

The morphological characters as well as the ontogenetic evidence from the above study bring about clearly that the super family *Diclidophoroidea* of Price (1936) is a heterogeneous assemblage of two distinct groups: *one group* with four clamp on each side and the functional haptor being formed from the anterior two-thirds of the larval haptor and the *other group* with the potentiality of unlimited addition of clamp units in metameric succession in which the functional haptor includes the anterior two-thirds of the haptor, though by far the greatest part is a newly formed entity in direct continuation forwards of the larval haptor. This latter group is represented by families *Microcotylidae*, *Axinidae* and *Gastrocotylidae*. The study on the juvenile and immature *Monaxine* Unnithan (1957) with reference to clamp addition by the author (in press) reveals that a maximum of four clamps are formed on each side of the haptor after which not only no more addition of clamps takes place on one side but the developed clamps on that side are also lost; thus suggesting that the development upto four clamps on each side is possibly recapitulating an identical character seen in the members of the superfamily *Diclidophoroidea* of Price (1936) except those of *Microcotylidae*, *Gastrocotylidae* and *Axinidae* of Unnithan (1957). Thus the evidences obtained from the studies justify the separation of these families from the superfamily *Diclidophoroidea* and the creation of a new superfamily to accommodate them. Though Unnithan (1957) has separated the above families from the superfamily *Diclidophoroidea*, he has not assigned any valid reasons. Instead he has created a new superfamily *Microcotyloidea* to accommodate them and has given the superfamily diagnostic characters also.

In view of the present observations, therefore, the diagnosis of the super-families have to be emended to include characters besides those mentioned by Price (1936) and Unnithan (1957) for *Diclidophoroidea* and *Microcotyloidea* respectively.

Diclidophoroidea emend Price 1936 *partim*

Polyopisthocotylea in which the functional haptor is contributed by the anterior two-thirds of the larval haptor. The number of clamps is limited to four on each side of the functional haptor. A pair of buccal pouches of prismatic fibres usually occur within the mouth and a genito-intestinal canal is probably always present. Parasites of gills of fishes or some times of crustacea within the mouth of fishes.

It includes the families *Mazocraeidae*, *Chimaericolidae*, *Discocotylidae*, *Hexostomidae*, *Diclidophoridae*, *Choricotylidae* and *Allodiscocotylidae* of Tripathi (1959).

Microcotyloidea emend Unnithan 1957 *Diclidophoroidea* Price 1936 *partim*

Polyopisthocotylea in which the functional haptor includes the anterior two-thirds of the larval haptor and by far the greater part of the adult haptor is a newly formed entity, in direct continuation forwards of the larval haptor and has the potentiality of unlimited addition of clamp-units in metameric succession. Haptoral units (clamps) not necessarily in pairs, present on both sides or on one side, either the right or the left. Tendency to either primary or secondary growth inhibition of one side of the hind body and haptor. In some only haptoral axis, other times there is a modification in size or shape of simple clamps leading to their conversion

into armed suckers and thence to asymetry and the addition of new sclerites. Total inhibition of the haptor axis may be present even from both sides of the haptor. Ovary in front of the testes. A pair of buccal pouches of prismatic fibres usually occur within the mouth and genito-intestinal canal is always present. Parasites of gills of fishes.

It includes the families Microcotylidae, Axinidae and Gastrocotylidae.

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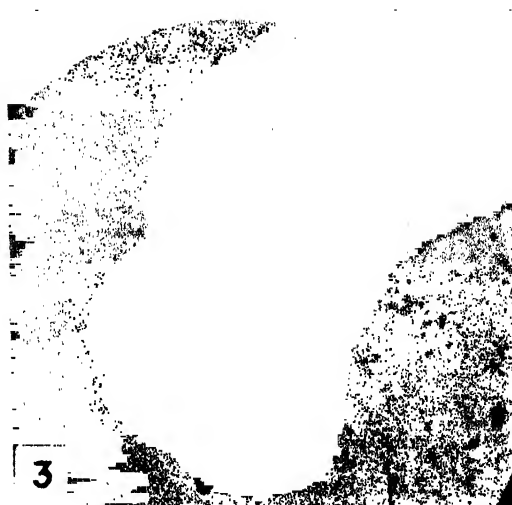
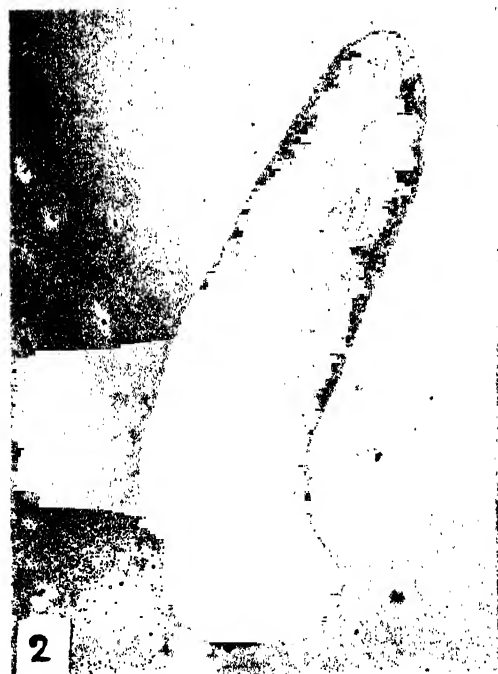
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EXPLANATION OF PLATE XXI

- Fig. 1. Adult specimen of *Heteraxine indica* showing the marginal hooklets $\times 260$.
- Fig. 2. Post-oncomiracidial larva of *Pricea* spp. $\times 150$.
- Fig. 3. Unmodified haptor of the post-oncomiracidial larva of *Pricea* spp. $\times 260$.
- Fig. 4. Haptor of the post-oncomiracidial larva of *Pricea* spp. showing the terminal lappet with anchors as well as posterior pair of marginal hooklets being pushed back $\times 150$.
- Fig. 5. The differentiation of the clamp anlagen in the region of the haptor of the post-oncomiracidial larva of *Pricea* spp. bearing the lateral marginal hooklets. $\times 155$.



PRELIMINARY STUDIES OF LEAF MORPHOGENESIS IN SOME VARIETIES OF RICE PLANT (*ORYZA SATIVA* L.)

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ABSTRACT

Three varieties of rice plants, *F.R.13A* (aman), *Satika* (aus) and *C.B.I.* (boro) were grown as broadcast paddy for the morphogenetic studies of the leaves borne by the main culm.

The varieties show differences in (i) the number of foliar leaves on main culm, (ii) the relative lengths of leaf sheath and leaf blade and (iii) the sequences of the ratios between the lengths of leaf sheath and leaf blade (S/B) in all the successive mature leaves.

The graphical representation of the S/B ratios indicates that the culm elongation phase in each variety was accompanied by a definite fall in the ratios. Ear formation is evident much earlier from the higher S/B ratio of the leaf developing just before the terminal ear leaf.

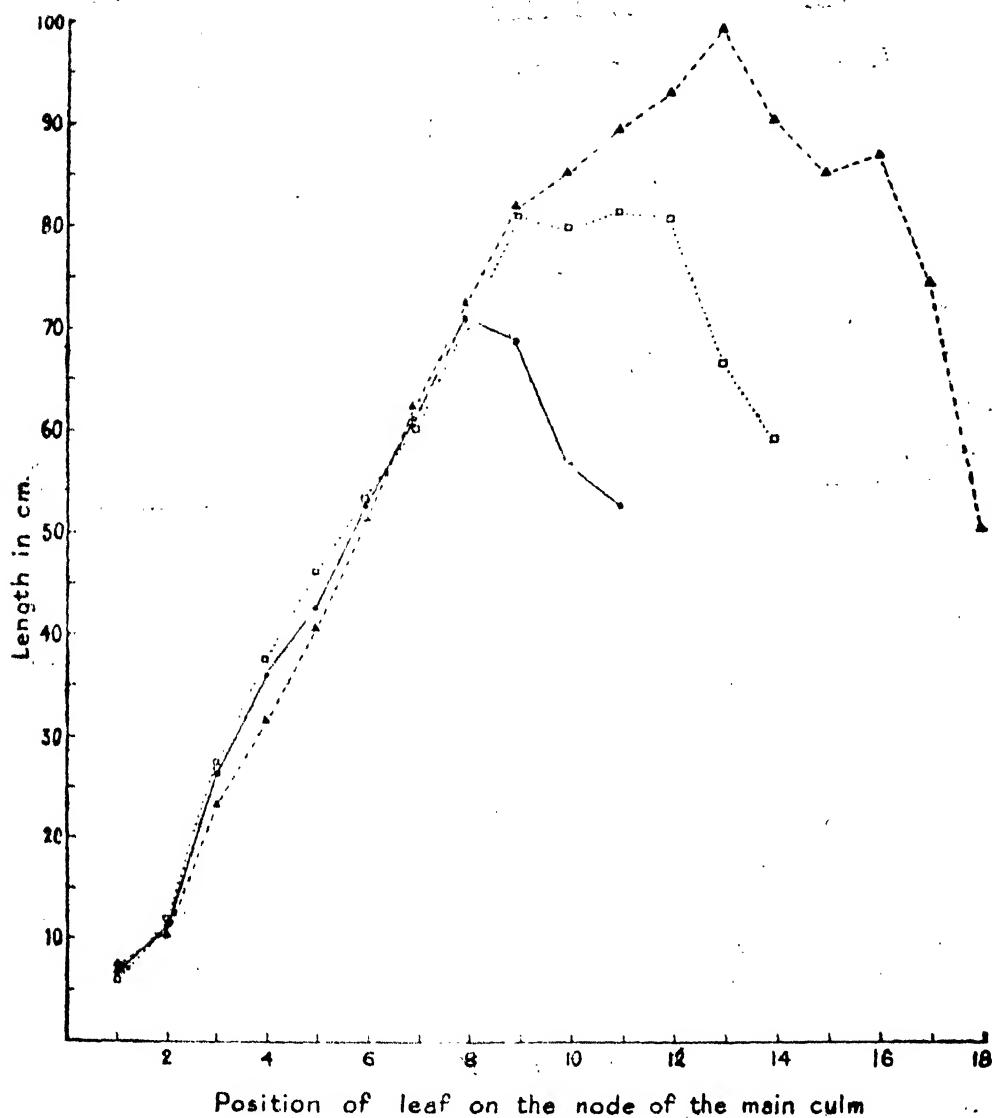
INTRODUCTION

The paddy varieties in Bengal are usually classified into three agricultural groups, viz., aman (winter), aus (autumn) and boro (spring) according to the season when it is harvested. Different vegetative characters are taken into consideration for describing the individual groups and varieties of rice, but little importance has so far been given to the behaviour of the plants with regard to the ratio of the length of leaf sheath to that of leaf blade, (S/B). Arashi and Eguchi (1954) studied the growth of leaf sheath and leaf blade of rice plant in relation to the life duration of the leaves. Recently Kaufman (1959b) has reported the trends of leaf growth in a rice variety, *Caloro*, but his observations are based on inadequate number of measurements. In order to study the sequences of S/B ratios in all the successive mature leaves of different varieties of rice and their relationship with certain important developmental stages of the plants, the present investigation with one variety of aman, aus and boro each was undertaken.

MATERIALS AND METHODS

Seeds of *F.R. 13A* (aman), *Satika* (aus) and *C.B. I* (boro), collected from the State Rice Research Station, Chinsurah, (West Bengal), were sown as broadcast paddy in big earthen pots on the 10th June, 1959. Germination was normal and the plants were allowed to grow under usual conditions. Almost every week the plants of each variety were separately collected at random to measure the length of leaf sheath and blade, and to note, amongst others, the phases of internode elongation and inception of ear primordium. These observations were continued upto the harvesting time. The individual length of the mature leaves, leaf sheaths and leaf blades as indicated in the graphs represented an average of about 21 measurements.

The leaves were numbered acropetally from the base upwards by designating the oldest first-formed leaf as 1, and the successively younger ones in the main culm as 2, 3, etc.



TEXT-FIG. 1.

Fig. 1. Relation of mean length of the successive mature leaves in cm. of F.R.13A (▲), Sanka (●) and U.B.I. (□).

OBSERVATIONS

The junction of sheath and blade in the rice leaf is generally marked by the presence of a thin scale-like ligule and a pair of sickles (Saha, 1952). The first leaf or prophyll is sheathing and non-laminate. It may be compared with leaf sheath from the presence of well-developed cross-connections between two contiguous longitudinal vascular strands. Grist (1953) describes prophyll as a bladeless leaf. Every axillary bud developing into a tiller is also associated at the beginning with such a leaf. The second and later-formed leaves evidently bear a blade each. The last vegetative leaf on each stem or culm is known as boot-, ear- or flag-leaf.

The number of leaves coincided with that of nodes on the main culm and was recorded to be 18, 11 and 14 in *F.R. 13A*, *Satika* and *C.B. I* respectively. In both *Satika* and *C.B. I* the leaf lengths increased almost definitely upto the leaf 4th from the terminal ear leaf when the stage of initiation of ear primordium began; in *F.R. 13A* the increase continued upto the leaf 6th from the ear leaf. In all the three plants, however, leaf lengths increased upto the stage when about three-fourths of the total number of leaves to be formed had developed. (Fig. 1).

A large number of nodes with very short internodes remained crowded at the basal portion of the culm, constituting a "hard culm base" (Katyama, 1931). The culm base was found to consist mostly of 10, 7 and 8 nodes in *F.R. 13A*, *Satika* and *C.B. I* respectively; extension of internodes subsequently started after the individual last nodes of the culm base, forming 8, 4 and 6 elongated internodes respectively.

The sheaths in almost all the three varieties continued to increase in length till the beginning of internode-elongation when their lengths decreased gradually. The length of leaf blades, however, showed a gradual rise from the 2nd to the 8th leaf in *Satika* and to the 11th leaf in *C.B. I* i.e., 4th leaf from the terminal one. In both of them, with the formation of long internodes the length of the leaf blade recorded a sudden jump and continued to rise till a fall was noted in the last three leaves (Fig. 2). As maturity advanced, the early-formed lower leaves began to die and often some of the blades were found to be damaged or injured; nevertheless, from the sequences of lengths of leaf blades and leaf sheaths it could be possible to identify as to which nodes they belonged.

The graphical representation of the sequences of the S/B ratios of the successive leaves in *F.R. 13A*, *Satika* and *C.B. I* showed two peaks, one at the 2nd, i.e., the first laminate leaf and the other at the last terminal ear leaf. In case of the leaves developing in the intermediate phase, the ratios, however, indicated comparatively low values (Fig. 3).

The S/B ratio of the 2nd leaf of the main culm was usually more than unity in *Satika* and *C.B. I* and slightly less in *F.R. 13A*. In all the successive later-formed leaves except the ear leaf the ratios were, however, far less than unity.

In *Satika* and *C.B. I*, the S/B ratios increased distinctly from the 4th leaf onwards upto the leaf developing before the formation of long internodes, but with the initiation of internode elongation they showed a definite and gradual fall upto the 8th and 11th leaf, respectively, i.e., 4th leaf from the terminal ear leaf. In *F.R. 13A* the rise and fall of such ratios before and after the starting of culm elongation were, however, not so evident.

The S/B ratios in the 10th leaf of *Satika* and the 13th leaf of *C.B. I* (i.e., 2nd leaf from the terminal one) showed a sudden rise from those of the leaves developing in the culm elongation phase, when the ear primordium was noted to have already developed fairly distinctly. In the ear leaf of *Satika* and *C.B. I*, the S/B ratio was slightly less than unity. The formation of ear or panicle becomes apparent from the terminal ear leaf having blade relatively shorter and broader; but evidences of ear formation could be noted even much earlier from the size variation of the leaf developing just before the terminal ear leaf.

DISCUSSION AND CONCLUSION

The present morphogenetic investigation on the three varieties of rice namely, *F.R. 13A* (aman), *Satika* (aus) and *C.B. I* (boro) shows that in these varieties there are differences in (i) the number of foliar leaves on the main culm, (ii) the relative lengths of leaf sheaths and of leaf blades, and (iii) the sequences of the ratios between

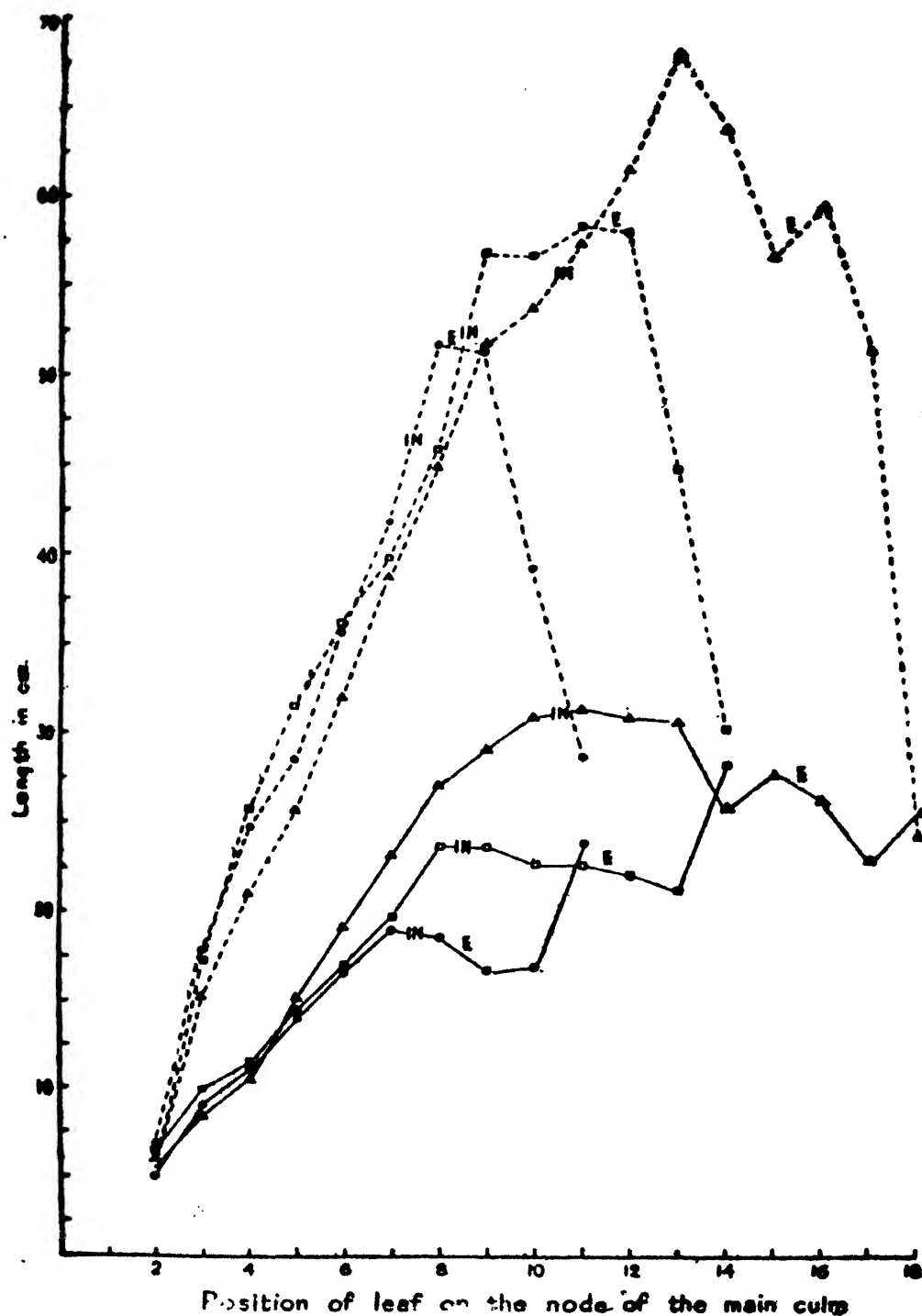
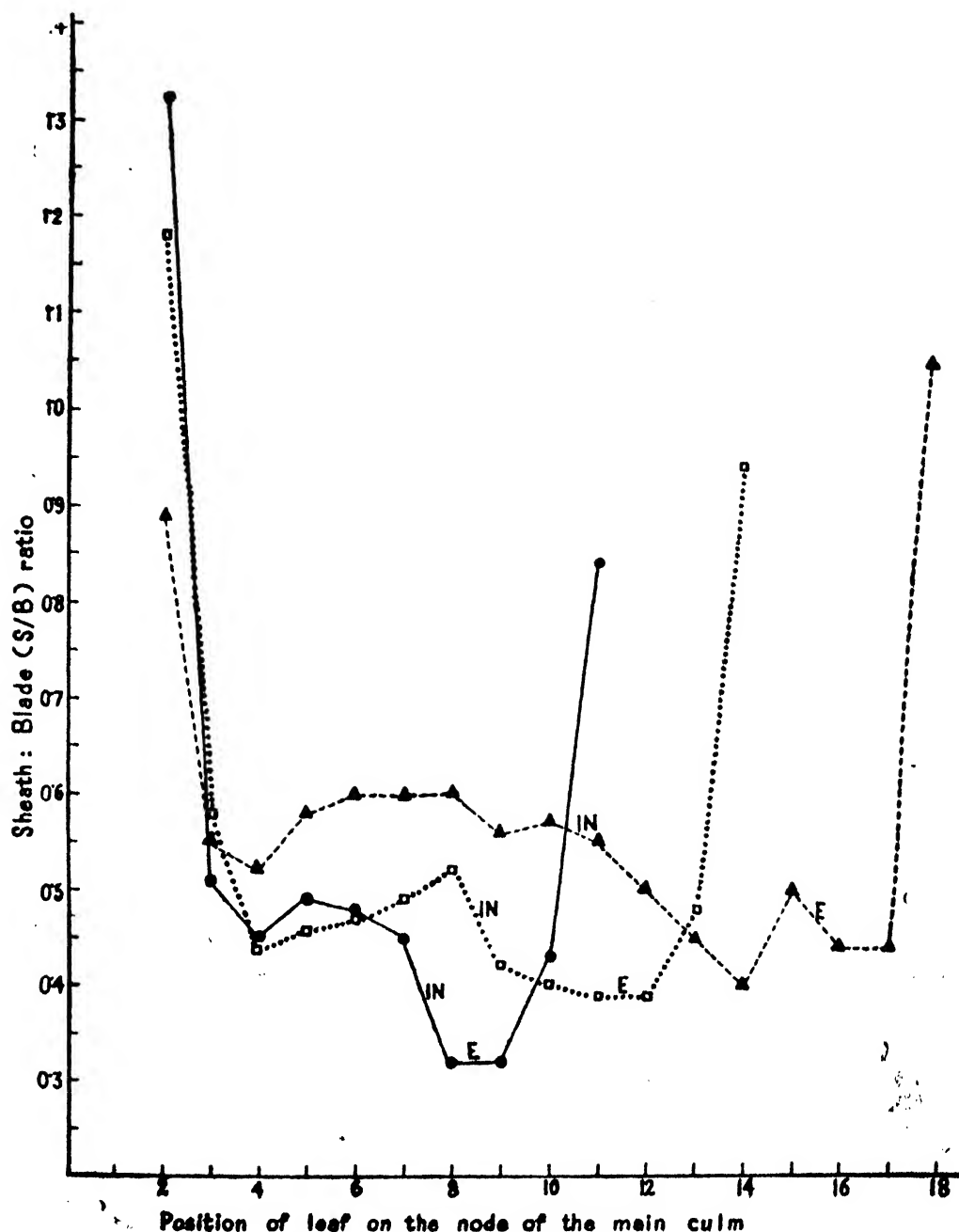


Fig. 2. Relation of mean length of the successive mature leaf sheath (—) and leaf blade (---) in cm. of the second to ear leaves of F.R. 13A (Δ), Sasika (\odot) and O.B.I. (\square). E and IN indicate respectively stage of ear initiation and that of internode elongation.



TEXT-FIG. 3.

Fig. 3. Sequences of the S/B ratios in the successive mature leaves of *F.R.13A* (Δ), *Satika* (\bullet) and *C.B.I.* (\square) E and IN indicate respectively stage of ear initiation and that of internode elongation.

the lengths of leaf sheath and leaf blade (S/B) in all the successive mature leaves. Interestingly enough, this S/B ratio indicates the development of internode elongation and that of ear initiation.

The leaf lengths in rice plant increase gradually upto the stage when almost three-fourths of the total number of leaves have been formed. This phenomenon of changes in size as seen in the successive leaves formed during the development is indicated to be associated with the usual homoblastic development (Goebel, 1905). Arashi and Eguchi (1954) have also noted the variation of life duration of each rice leaf according to its position on the culm, the later-formed upper leaves having longer life. The gradual increase in leaf size may, however, be correlated with the progressive elaboration of the shoot apical meristem during the development of the plant (Saha, 1954; Kaufman, 1959a). In maize Abbe *et al.*, (1941) have also correlated the progressive broadening of the leaves with the growth of the plant showing comparable increase in the width of the shoot apex. In dealing with the formation of sori in Onocleoid and Blechnoid ferns, Goebel (1930) has referred to the importance of correlative development. This phenomenon may be involved in the changes of variation in leaf size of rice plant according to the age and conditions of plant growth. From another angle of views, changes in morphology from leaf to leaf are again attributed to a function of physiological age of the plant (Krenke, 1940) and to some process of ageing in the meristem, nutritional changes being possibly involved (Wardlaw, 1945, 1946; Ashby and Wangermann, 1950).

The sheathing form of prophyll and relatively greater S/B ratios of 2nd and 3rd leaves due to relatively more growth of sheath may indicate the result of their primordial forms being reported to be present in the seeds, which are far advanced at germination (Saha, 1956). The arrest of lamina growth was progressively delayed in the successively younger ones during vegetative growth, when a higher concentration of nitrogen, amino acid and amide nitrogen, and a lesser carbohydrate content were noted (Nagai, 1959).

The relatively shorter sizes of the leaves with higher S/B ratios in the last three leaves in all the three varieties probably result from the ear initiation during their growth and elongation: because there are in a rice apex three leaf primordia in their early stages of ontogeny at the time when the apex starts differentiating into an ear primordium (Akimoto and Togari, 1939). During the period of ear formation a greater amount of energy is known to be consumed in the development of the ear and the carbon-nitrogen ratio of the plants showed a decided drop (Nagai, 1959).

Formation of long internodes at the beginning is not externally noticeable, but the sudden rise in blade length and a sharp change in S/B ratio fairly indicate the initiation of culm elongation. Extension of internodes mostly occurred after the 10th leaf in *F.R. 13A*, the 7th in *Satika* and the 8th in *C.B. I* forming respectively 8, 4 and 6 elongated internodes. Greater number of long internodes with longer elongation phase may point to the flood resistance capacity of *F.R. 13A*, the length of culm being practically dependent on the number and length of long internodes.

In connection with the studies on the relation of the organo-morphological expression of the vegetative with reproductive organs, Matsushima *et al.* (1954), have indicated the stage of growth in the tiller in terms of "foliar age index" (i), which is expressed in terms of the percentage ratio of the number of leaf (n) and the total number of leaves (N) on the main culm which is fairly invariable as a varietal character ($i = n/N \times 100$). For example, if the index is 50, it is indicated that the culm is in a state in which half the number of the total leaves to be formed have already developed. Such indices at the time of initiation of long internode in *F.R. 13A*, *Satika* and *C.B. I* are roughly 56, 64 and 57 respectively showing that internode elongation may begin when slightly more than half of the total leaves of the plant had formed.

Ramiah and Rao (1953) mention that during the tillering phase and until the formation of primordium ear, a rice plant does not show any elongated internodes,

exception being the deep water rices; vigorous shoot growth starts only with the commencement of ear formation. But in the present study elongation of internode does not strictly show direct relation with ear development. It has been noted that the initiation of ear primordium occurs at a stage when the 4th leaf from the terminal one has almost completed development, i.e., at the stage when 5, 1 and 3 long internodes have already been formed in *F.R. 13A*, *Satika* and *C.B. 1* respectively. The differentiation of the shoot apex of rice into the primordium of panicle or ear is also reported to start at a stage when the 4th leaf from the last ear leaf has developed fairly distinctly (Akimoto and Togari, 1939).

In the present study the culm elongation phase has been seen to be accompanied by a definite fall in Sheath/Blade ratios, when the culm forms more than half of the total number of leaves in *F.R. 13A*, *Satika* and *C.B. 1*. A sharp rise in such ratios towards the end of plant life indicates the initiation of ear primordia in almost all of them.

Further comparative investigations in the other rice varieties and in those already studied under variable conditions are in progress.

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MORPHOLOGICAL DESCRIPTIONS OF A NEW GENUS *NEOMICROCOTYLE*
AND THREE NEW SPECIES OF THE GENUS *PROTOMICROCOTYLE*
(MONOGENEA) WITH A DISCUSSION ON THEIR TAXONOMIC
POSITION*

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ABSTRACT

Neomicrocotyle indicus n.g. et n.sp., and three new species of *Protomicrocotyle* viz., *P. madrasensis*, *P. minutum* and *P. mannarensis* are described.

Taxonomic position of the genus *Protomicrocotyle* and its related groups is discussed. Opinion is expressed in favour of the revival of the subfamily *Protomicrocotylinae* as well as its transfer to the family *Allodiscocotylidae*. The subfamily *Bilateracotylinae*, provisionally created by Chauhan (1953) is revived here for the genus *Bilateracotyle* Chauhan (1945). A new subfamily *Neomicrocotylinae* is created here for the new genus *Neomicrocotyle*. The two subfamilies *Bilateracotylinae* and *Neomicrocotylinae* are provisionally included in the family *Allodiscocotylidae* which necessitated an amending of the family diagnosis.

INTRODUCTION

In the course of the investigation on the trematode parasites of fishes from Mandapam, the author collected from some carangid fishes monogenetic trematodes closely resembling the genus *Protomicrocotyle* Johnston and Tiegs (1922) but having microcotylid clamps as well as those belonging to the genus *Protomicrocotyle* but different from the known species. Because of the differing views expressed with regard to the systematic position of the genus and the subfamily constituting it (Johnston and Tiegs, 1922), Sproston (1946), Chauhan (1953), Hargis (1957), and [Tripathi (1959)], it is considered here to be of some interest to present a detailed study of these forms possessing microcotylid and gastrocotylid types of clamps. Incidentally the genus *Protomicrocotyle* is recorded for the first time from India. The type specimens of the new genus and the new species are deposited with the Zoological Survey of India, Calcutta and the paratypes are in the collections of the author.

MATERIAL AND METHODS

The material used in this study was obtained from the gills of *Caranx sexfasciatus* Quoy & Gaimard and *Caranx affinis* Rupp. Observations were made on live specimens and were fixed under cover slip pressure in warm 7% formalin. They were stained in Mayer's paracarmine, and in Ehrlich's haematoxylin, differentiated in acid alcohol and blued with ammonia alcohol (70%). Permanent mounts were prepared and measurements were made from them.

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Neomicrocotyle indicus n.g. et n.sp.

(Figs. 1-5)

Out of ten *Curanx sexfasciatus* Quoy & Gaimard examined, five specimens were found to be infected and yielded eight parasites.

The body is elongate and flattened. Its length ranges from 3.59 mm. to 4.67 mm. and the largest width across the oviducal field is from 0.80 mm. to 1.15 mm. The body narrows gradually anteriorward ending pointedly anteriorly: its width across the level of the intestinal bifurcation is half the maximum width and is one fourth across the level of the male genital pore which is situated half way between the intestinal bifurcation and the anterior extremity of the body. Posterior to the oviducal field the body narrows, its width across the anterior border of the lobed side of the body is three fourths and at the junction of the body with the dumb-bell shaped haptor is one third the maximum width.

The posterior extremity of the body is lobed and is either on the right or left of the worm and the lobed portion extends to a length of 0.29 mm. to 0.60 mm. from the junction of the body with the dumb-bell shaped haptor. Four sessile clamps, arranged in an irregular vertical series, occur on the lobed side of the body. The clamps are of the *microcotylid* type. The clamp-size ranges from $38 \times 36\mu$ to $52 \times 72\mu$. The body ends in a transversely extended dumb-bell shaped lobe. Its width ranges from 0.63 mm. to 0.74 mm. and its length at the expanded region is 0.22 mm. to 0.28 mm., at the middle region is 0.17 mm. to 0.26 mm. It has two pairs of anchors, the outer larger pair is 30μ to 35μ and the inner smaller pair is 17μ to 22μ . The posterior portion of the body and the dumb-bell shaped haptor show transverse striations which give a spiny appearance to the surface of the worm at this region and they are directed forwards.

Mouth is subterminal and ventral. Buccal suckers range in size from $29 \times 40\mu$ to $35 \times 61\mu$ and they are aseptate. The size of the pharynx ranges from $33 \times 17\mu$ to $39 \times 24\mu$. Intestinal bifurcation is situated at a point about one-fifth of the total length from the anterior end of the body with greater ramifications on to their outer sides. They terminate blindly anterior to the dumb-bell shaped haptor.

Testes numbering 140 to 164 are arranged in three to four rows on either side of the median line in the intercrural space and they are all anterior to the ovary. They occupy the middle third of the body. The extent and the number of testes are greater on the side of the worm having clamps. These two features were consistently seen in all the eight specimens examined. The size of the testis ranges from $20 \times 20\mu$ to $56 \times 56\mu$. Vas deferens takes a zig-zag course in the median line upto the level of the intestinal bifurcation. Beyond this level it is lateral, either on the left or right of the worm and in all cases it occurs on the side where the clamps also occur. Penis is armed with 24 hooks, arranged in a regular corona and its length ranges from 136μ to 155μ . A muscular bulbous ejaculatorius ($150 \times 175\mu$ to $185 \times 210\mu$) connects the vas deferens with the penis. Male pore is lateral, on the ventral side and is situated at a point two-thirds the length from the anterior extremity to the intestinal bifurcation.

Ovary and the oviduct occupy the anterior two-fifths of the hinder third of the body. The proximal end of the ovary is tubular and is bent in the form of 'U'. As their inner sides are so closely juxtaposed, this region gives roughly the appearance of a spherical mass. The measurements given relate to this spherical mass and its size is in the range of 0.17×0.16 mm. to 0.24×0.25 mm. It is situated close to the right caecum. Oviduct arises dorsal to the ovary and runs obliquely forward curving to the right. It extends posteriorly to about two-thirds the length of the oviducal field and makes a loop by curving to the left. The swollen end

of the oviduct is directed backwards. It is continued by a narrow duct which curves anterior to the ovary and on its left. It enlarges into the ootype which is surrounded by the shell glands. Beyond the ootype the oviduct is continued as the uterus which is median upto the intestinal bifurcation. From this point onwards it is lateral, either on the right side or left side of the worm and is always on the side where the male pore is situated. Uterine pore is ventral and is situated posterior to the male pore.

Vitelline follicles commence from the level of the intestinal bifurcation and extend as two lateral bands to the posterior end of the body. The transverse vitelline ducts originate about the level of the anterior fifth of the oviducal field and the common vitelline duct extends to a length equal to two fifth the length of the oviducal field. The common vitelline duct and the genito-intestinal canal open at the ootype. Vaginal pore is submarginal and in all specimens examined, it is on the side opposite to that where the male pore and the clamps occur. Its position is mid-way between the male pore and the intestinal bifurcation. It leads to an enlarged muscular sac. Its inner wall is lined with numerous short conical projections. It is continued posteriorwards as a narrow duct close to the inner side of the left caecum and opens just posterior to the ootype. About half-way down the oviducal field the vaginal canal has an enlargement serving as the receptaculum seminis; its size is from $38 \times 16\mu$ to $40 \times 21\mu$.

Remarks: The present form described resembles the genus *Protomicrocotyle* in the general shape; in the possession of an irregular vertical series of four clamps on the lobed side of the body; pre-ovarial testes, lateral vagina and armed penis but it differs from *Protomicrocotyle* by the possession of clamps of the microcotylid type instead of the gastrocotylid type present in *Protomicrocotyle*. To assign the present form in the genus *Protomicrocotyle* would entail the inclusion in one and the same genus members having both gastrocotylid and microcotylid types of clamps. Hence a new genus *Neomicrocotyle* is created to include the present form and its generic diagnosis is as follows:—*Neomicrocotylinae* (*vide infra*) having 4 clamps of microcotylid type, arranged in an irregular vertical series on the lobed side of the body; the male and vaginal pores lateral; uterus median upto the level of the intestinal bifurcation and lateral beyond, on the same side as that of the male pore whereas the vaginal pore is on the side opposite to that having the male and uterine pores.

A similar observation on the presence of microcotylid and gastrocotylid types of clamps in closely related genera *Bilateracotyle* Chauhan (1945, 1953) and *Bilateracotylodes* Ramalingam (In press) respectively has been recorded by the present author.

Protomicrocotyle pacifica Meserve (1938) possesses microcotylid clamps which necessitates its inclusion in the new genus and a new combination is made for it namely *Neomicrocotyle pacifica* (Meserve, 1938). The present form resembles *N. pacifica* (Meserve, 1938) in the possession of three to four rows of testes on each side of the median line, of vagina, vas deferens and uterus all of which are lateral in position. It differs from *N. pacifica* in possessing 24 uniform sized hooks connected with the male genitalia. These hooks are arranged in a regular corona and they measure from 136μ to 155μ . But in *N. pacifica* the genital atrium is armed with 20 hooks, ten of which are shorter and they measure 108μ to 120μ and ten are longer measuring 168μ to 184μ . In addition to the above difference the clamps of *N. indicus* are much smaller, nearly half the size of those in *N. pacifica* in the nearly same sized specimens examined. Hence it is described as a new species *N. indicus* and its specific diagnosis is as follows:—*Neomicrocotyle* with 24 uniform sized hooks ranging from 136μ to 155μ connected with the male genitalia; length of the worm from 3.59 mm. to 4.67 mm. and its largest width is from 0.80 mm. to 1.15 mm.; clamp-size ranges from $38 \times 36\mu$ to $52 \times 72\mu$; with 2 pairs

anchors, outer pair from 30μ to 50μ long; and inner pair 17μ to 22μ ; testes number from 140 to 164 and are pre-ovarial; ovary size is from 0.17×0.16 mm. to 0.24×0.25 mm.

Protomicrocotyle madrasensis n.sp.

(Figs. 6-9)

From a collection of two *Caranx affinis* Rupp. examined a single worm was obtained from the gills.

It is 2.39 mm. long and the maximum width across the oviducal field is 0.41 mm. The lobed portion at the posterior end the body is on the right side of the worm and extends to a length of 0.22 mm. Four sessile clamps are present on the lobed side of the body. The clamps have two accessory pieces meeting in the form of a 'V' characteristic of the members of the family Gastrocotylidae. The clamp size is $32 \times 48\mu$ to $33 \times 56\mu$. At the posterior end of the body is the dumb-bell shaped haptor. It is 0.24 mm. broad, its length at either ends is 0.12 mm. and in the middle is 0.11 mm. It has three pairs of anchors, the outer larger is 28μ , the inner smaller pair is 17μ and the third pair is situated in between the two dissimilar pairs of anchors. Its length is 9μ . The shape of the outer pair and the inner pairs of anchors are similar to those present in *Neomicrocotyle indicus* n.g. et n.sp.

Mouth is subterminal and ventral. The size of the buccal sucker is $24 \times 36\mu$ and it is aseptate. The size of the pharynx is $41 \times 38\mu$. Intestinal bifurcation is situated at a point one-fifth of the total length from the anterior end of the body.

Testes numbering 64 are arranged in two rows on either side of the median line in the intercrural space and they are all anterior to the ovary. They occupy the middle third of the body. The size of the testes ranges from $33 \times 38\mu$ to $58 \times 81\mu$. Vas deferens takes a zig-zag course in the median line. Penis is armed with 20 hooks and they are arranged in a regular corona. It is situated at a point five-sixth the length from the anterior extremity to the intestinal bifurcation. Each hook is 12μ long. In addition to this the genital atrium is armed with four hooks, two on either side of the penis corona and each hook is 33μ long. A muscular bulbous ejaculatorius connects the vas deferens with the penis corona. Male pore is median on the ventral side and situated immediately in front of the penis corona.

Ovary and the oviduct occupy the anterior half of the hinder third of the body. The size of the ovary is $127\mu \times 178\mu$. The distal end of the oviduct is directed backwards and is continued by a narrow duct which curves anterior to the ovary to the left. It enlarges into the ootype which is surrounded by the shell gland. Beyond the ootype the oviduct is continued as the uterus which is median. It opens on the ventral side a little posterior to the male pore. Vaginal pore is sub-marginal and is on the side opposite to that having the clamps. The vaginal pore leads to a muscular sac whose inner wall is lined with conical projections resembling spines. They are directed forwards and their length ranges from 21μ to 31μ . From the sac a narrow duct leads posteriorly close to the inner side of the left caecum. About half way down the oviducal field the vaginal canal shows an enlargement serving as the receptaculum seminis. Its size is $28 \times 14\mu$. Further down it joins the oviduct posterior to the ootype.

Remarks: *Lethacotyle* Manter and Prince (1953) shows striking resemblance to the genus *Protomicrocotyle* but differs from it in the complete absence of clamps. Since only two specimens were used by the above authors for the original description, it might be that the clamps in these two specimens were lost while handling. As these authors discount its possibility, it is felt better to leave it till more evidences are set forth to confirm this condition.

The genus *Protomicrocotyle* includes the following species :—*P. mirabilis* (MacCallum, 1918), Johnston and Tiegs (1922), and *P. celebesensis* Yamaguti (1953). The present form described agrees with *P. mirabilis* and *P. celebesensis* by the possession of gastrocotylid clamps. Since the description of *P. mirabilis* regarding the taxonomically important characters, other than the clamp structure, is meagre and needs a redescription as Hargis (1957) puts it, a comparison with *P. celebesensis* only is made here. *P. madrasensis* agrees with *P. celebesensis* in the median position of the genital armature and in the number of testes but differs from it in the possession of two different sized hooks connected with the male genitalia. Four of these hooks are longer each measuring 33μ and 20 short hooks each 12μ long. Hence it is described as a new species and its diagnosis is as follows : *Protomicrocotyle* having two different sized hooks connected with the male genitalia ; four longer each 33μ long and 20 shorter each 12μ long ; male genital pore median ; the length of the worm is 2.39 mm. and the largest width is 0.41 mm. clamp-size $32 \times 48\mu$ to $33 \times 56\mu$; with 3 pairs of anchors, the outer pair 28μ , the inner pair 17μ and the middle pair 9μ long ; testes number 64 and ovary size $127\mu \times 178\mu$.

Protomicrocotyle minutum n.sp.

(Figs. 10–12)

A single specimen was obtained from a collection of two *Caranx sexfasciatus* Quoy & Gaimard, examined.

The body is elongate with the sides of the body nearly parallel from the beginning of the testes to the level of the last clamp. The width of the body narrows gradually, in front of testes, it is three fourths of the maximum width ; across the intestinal bifurcation it is about half the maximum width. The worm measures 1.50 mm. and its maximum width is 0.30 mm. The lobed portion at the posterior extremity of the body is on the right side of the worm and extends to a length of 0.18 mm. Four sessile clamps (gastrocotylid type) are present on the lobed side of the body, each one measuring $33\mu \times 52\mu$. At the posterior end of the body is the transversely extended lobe which is oval in shape. Its width is 0.36 mm. and its length is 0.14 mm. It has two pairs of anchors, the outer larger pair is 24μ and the inner smaller pair is 14μ . Their shape is similar to those present in *Neomicrocotyle indicus* n.g. etn.sp.

Mouth is subterminal and ventral. The size of the buccal sucker is $32\mu \times 63\mu$ and it is aseptate. The size of the pharynx is $39\mu \times 39\mu$. Oesophagus bifurcates midway between the anterior extremity and the beginning of the testicular region.

Testes numbering 34 are arranged in two lateral rows. They occupy the posterior two thirds of the middle third of the body. Size of the testes ranges from $14 \times 14\mu$ to $34 \times 44\mu$. Vas deferens is median upto the level of the intestinal bifurcation and takes a zig-zag course. Beyond this level it is lateral on the right side of the worm. Penis is armed with 8 hooks, arranged in a regular corona and its length is 43μ . A muscular bulbous ejaculatorius ($39\mu \times 39\mu$) connects the vas deferens with the penis. Male pore is situated about half way behind from the anterior extremity to the intestinal bifurcation.

Ovary and oviduct occupy the proximal third of hinder third of the body. Size of the ovary is $49\mu \times 36\mu$. The distal end of the oviduct is directed backwards and is continued by a narrow duct. It curves to the left anterior to the ovary and enlarges into the ootype which is surrounded by shell glands. The uterus is median upto the level of the intestinal bifurcation and beyond it is lateral, on the right side. It opens on the ventral side posterior to the male pore.

Vagina is lateral on the left side opposite to that having the male pore and clamps. Vaginal pore is submarginal and is situated midway between the level

of the male pore and the intestinal bifurcation. The pore leads to an enlarged muscular sac whose inner wall has 20 prominent conical projections of which 10 are distinctly shorter and they alternate with the longer ones. They measure 15μ and 29μ long respectively. From the sac a narrow duct leads posteriorly close to the inner side of the left caecum. About half way down the oviducal field the vaginal canal shows an enlargement serving as the receptaculum seminis. Its size is $23\mu \times 9\mu$. Further it is continued as a narrow duct and opens at the oviduct posterior to the ootype.

Protomicrocotyle minutum differs from *P. madrasensis* in the possession of the male opening which is lateral and the penis is armed with only 8 uniform sized hooks, each 43μ long. Besides, in *P. minutum* the vaginal sac has ten large conical projections each 29μ long and alternating with them are ten shorter conical projections each 15μ long. Hence it is described as a new species and its specific diagnosis is as follows: *Protomicrocotyle* having only 8 hooks connected with the male genitalia, all of uniform size, each 43μ long; male genital pore lateral to the median line; vaginal sac has 20 conical cuticularized projections of which ten are longer each 29μ long and ten shorter each 15μ long; the length of the worm is 1.50 mm. and its greatest width is 0.30 mm.; clamp size is $33\mu \times 52\mu$; with 2 pairs of anchors, the outer pair is 24μ and the inner pair 14μ long; testes number 34 and ovary size $49\mu \times 36\mu$.

Protomicrocotyle mannarensis n.sp.

(Figs. 13-15)

Ten specimens were obtained on three occasions from out of ten *Caranx sexfasciatus* Quoy & Gaimard examined.

The worm measures from 2.88 mm. to 3.64 mm.; its width across the oviducal field ranges from 0.48 mm. to 1.14 mm. The lobed portion at the posterior extremity of the body is either on the left or right of the worm and extends to a length of 0.27 mm. to 0.39 mm. Four sessile clamps of the gastrocotylid type are present on the lobed side of the body and their size ranges from $30 \times 46\mu$ to $38 \times 71\mu$. A transversely extended lobe oval in shape is present at the posterior end of the body. Its width ranges from 0.32 mm. to 0.44 mm. and its length is 0.13 mm. to 0.17 mm. It has three pairs of anchors, the outer larger pair is 33μ to 37μ and the inner smaller pair is 20μ to 22μ and the middle pair is 14μ to 17μ long. The shape of the outer and inner pairs of anchors are similar to those present in *Neomicrocotyle indicus* n.g. et n.sp.

Mouth is subterminal. Buccal suckers are aseptate and their size ranges from $23 \times 32\mu$ to $31 \times 42\mu$. The size of the pharynx ranges from $33 \times 35\mu$ to $41 \times 42\mu$. Intestinal bifurcation is situated at the hinder seventh of the anterior fifth of the worm.

Testes number ranges from 42 to 52 and they occupy the middle third of the body. Their size ranges from $21 \times 33\mu$ to $67 \times 181\mu$. The penis is armed with 24 hooks arranged in a regular corona and its length is from 21μ to 25μ . A muscular bulbous ejaculatorius connects the vas deferens with the penis. Its size ranges from $28 \times 25\mu$ to $36 \times 33\mu$. Male pore is median and is situated at a point two thirds the length from the anterior end to the intestinal bifurcation.

Ovary and the oviduct occupy the proximal half of the hinder third of the body. The size of the ovary ranges from $80 \times 60\mu$ to $161 \times 121\mu$. It is situated close to the right caecum. The distal end of the oviduct is directed backwards and is continued by a narrow duct which curves up anterior to the ovary. It enlarges into the ootype, which is surrounded by shell glands. Uterus is median throughout its course and opens on the ventral side posterior to the level of the

male pore. Vagina is either on the left or on the right side of the worm. In all the specimens examined, vaginal opening occurs on the side opposite to that having the clamps. Vaginal pore is submarginal and is situated midway between the level of the male pore and the intestinal bifurcation. The pore leads to an enlarged muscular sac. Its inner wall is lined with prominent conical projections and its length ranges from 17μ to 49μ . The distal portion of the projection is cuticularized and they are all directed forwards. From the sac a narrow duct leads posteriorly close to the inner side of the left caecum. The vaginal canal has an enlargement serving the function of receptaculum seminis. It is situated at a distance of about one third the length of the oviducal field from its anterior end. Its size ranges from $110 \times 35\mu$ to $126 \times 51\mu$. Further down it is continued as a narrow duct and opens at the oviduct posterior to the ootype.

Protomicrocotyle munuarensis differs from *P. madrasensis* in the possession of uniform sized hooks connected with the male genitalia in which respect it agrees with *P. minutum* and *P. celebesensis* as well. But it differs from *P. minutum* and agrees with *P. celebesensis* in the median position of the male genital opening. It differs from *P. celebesensis* in turn in the size and the number of penis hooks. Hence it is described as a new species and its specific diagnosis is as follows: *Protomicrocotyle* having 24 hooks connected with the male genitalia; all of uniform size, 21μ to 25μ long; male genital pore median; vaginal sac with many conical cuticularized projections, 17μ to 49μ long; the length of the worm is from 2.88 mm. to 3.64 mm. and its largest width is 0.48 mm. to 1.14 mm.; clamp size $30 \times 46\mu$ to $38 \times 71\mu$; with 3 pairs of anchors, the outer pair 33μ to 37μ , the inner pair 20μ to 22μ and the middle pair is 14μ to 17μ long; testes number 42 to 52 and ovary size is from $80 \times 60\mu$ to $161 \times 121\mu$.

Taxonomic considerations: Subfamily Protomicrocotylinae was created by Johnston and Tiegs (1922) to accommodate *Protomicrocotyle mirabilis* (MacCallum, 1918) and included it in the family Microcotylidae. Owing to the limited number of clamps in *Protomicrocotyle*, Sproston (1946) is of the opinion that the genus is not more closely related to the family Microcotylidae and preferred to group it in the family Discocotylidae even though according to her it means a heterogeneous assemblage. She assigned it under the subfamily Vallisiinae since *Vallisia* was the only genus then known to have the ovary wholly posterior to testes and the body axis bent at an angle with the result the subfamily Protomicrocotylinae became synonymous with Vallisiinae. But while concluding, she states that she regarded the above arrangement as a matter of convenience and a temporary measure until the knowledge of these aberrant forms is increased. Dawes (1947) did not recognize the subfamily Vallisiinae of Price (1943) which resulted in the inevitable transfer of the genus back to the family Microcotylidae. Chauhan (1953), on the basis of the clamp structure, retained the genus *Protomicrocotyle* in the family Microcotylidae, though by then one species of this genus viz., *P. celebesensis* Yamaguti (1953) is known to possess gastrocotylid clamps and assigned the genus under the subfamily Protomicrocotylinae. Since Chauhan's observations were not based on anatomical evidence, Hargis (1957) removed the genus to the family Gastrocotylidae on the basis of the presence of gastrocotylid clamps in the members of the type genus *Protomicrocotyle*. He also transferred Vallisiinae from the family Discocotylidae to Gastrocotylidae on the basis of the presence of gastrocotylid clamps in the type genus (*Vallisia* Perugia and Parona, 1896) constituting the subfamily and assigned the genus *Protomicrocotyle* Johnston and Tiegs (1922) under this subfamily. In it he included *Vallisiopsis* Subhadrappa (1951), *Allodiscocotyla* Yamaguti (1953), *Lethacotyle* Manter and Prince (1953) and *Pseudomazocraes* Caballero and Bravo (1955). Thus the review suggests that certain amount of ambiguity exists regarding the systematic position of the genus *Protomicrocotyle* and its related groups.

The study on the juvenile and immature *Monaxine* Unnithan (1957) (Fam : Axinidae) with regard to the clamp addition by the author (in press), reveals that a maximum of four clamps are formed on each side of the haptor after which not only no more addition takes place on one side but the developed clamps themselves on that side are also lost thus suggesting that the development upto four clamps on each side is possibly recapitulating an identical character seen in the members of the superfamily Dielidophoroidea of Price (1936) except those of Microcotylidae, Gastrocotylidae and Axinidae of Unnithan (1957). The study on the juvenile *Lithidiocotyle secunda* Tripathi (1954), *Kannaphallus univaginalis* Ramalingam (in press), on an adult *Heteraxine indica* Ramalingam (in press) and a long series of post-oncomiracidial larvae, juveniles of *Pricea* spp. by the author (in press) reveals that the members of the families Microcotylidae, Gastrocotylidae and Axinidae have the potentiality to add unlimited number of clamps and the functional haptor includes the anterior two thirds of the larval haptor though by far the greatest part is a newly formed entity in direct continuation forwards of the larval haptor whereas in the rest of the families, included in the superfamily Dielidophoroidea, with four clamps on each side, the functional haptor is formed from the anterior two thirds of the larval haptor. The evidences obtained seem to suggest that the inclusion of the above genera on the basis of the clamp structure alone into the families Microcotylidae and Gastrocotylidae is only extending their affinities too far. On the other hand the occurrence of microcotylid and gastrocotylid clamps in these might be that they represent parallel evolution in unrelated groups. Similar instances can be had in Monogenea itself, wherein identical characters have evolved in unrelated groups. For example, the cuticularized tubular penis described in *Kannaphallus* Unnithan (1957) is not uncommon in the members of the subfamilies Tetraonchinae and Diplectaninae of the family Dactylogyridae.

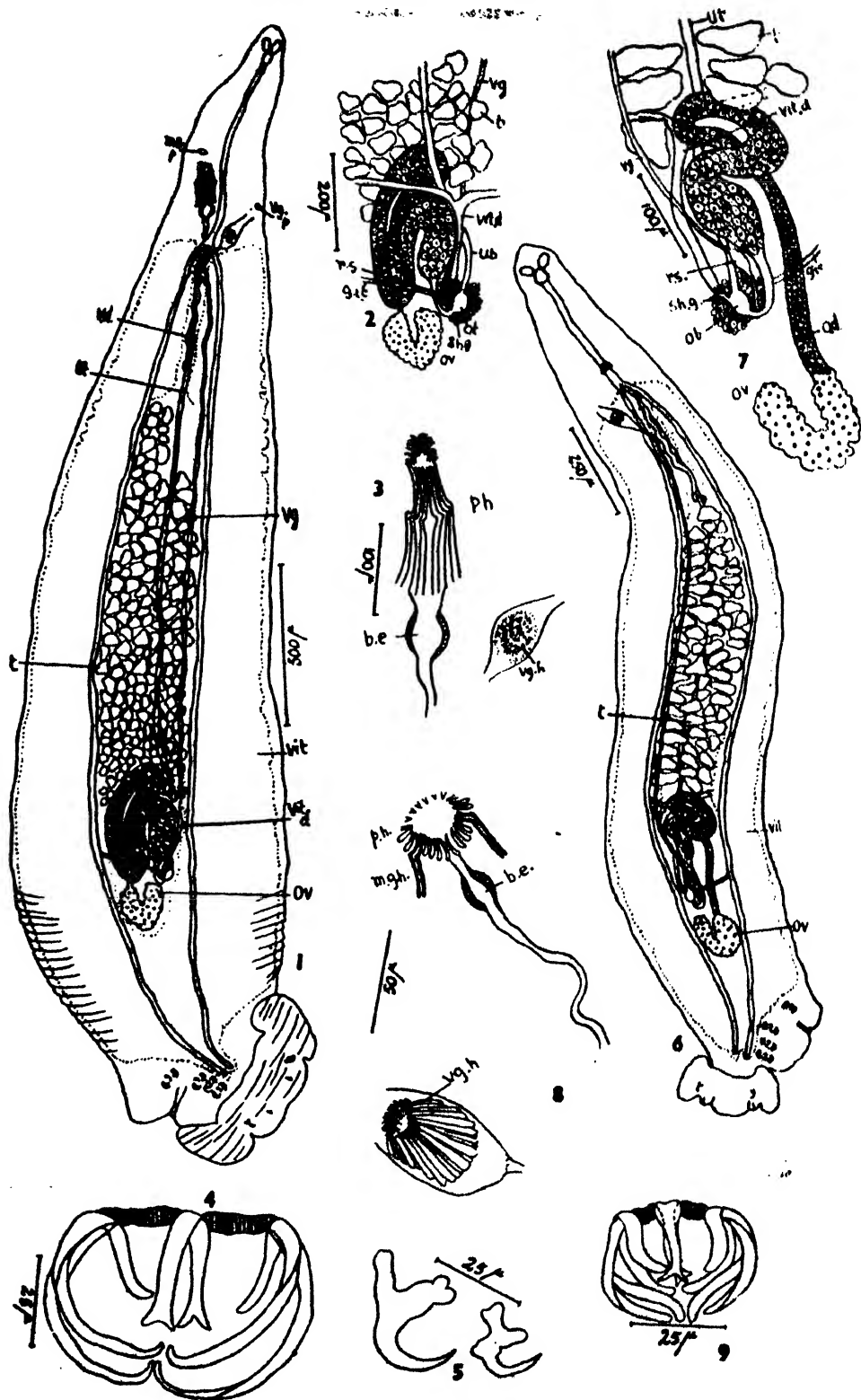
Thus from the evidences obtained it is felt unjustified to include the genera either in the family Microcotylidae or Gastrocotylidae and it is better to group them in a separate family. The author thus agrees with Tripathi (1959) who has created a new family Allodiscocotylidae to accommodate his genus *Gemmaecaputia* as well as *Allodiscocotyla*, *Vallisia*, *Vallisiopsis*, *Protomicrocotyle*, *Bilateracotyle* all having two common characters viz., pre-ovarial testes and gastrocotylid clamps (except *Bilateracotyle*) limited to a maximum of eight. The author has given his reasons in his paper on *Paragemmaecaputia* (in press) for agreeing with Tripathi for the creation of a new subfamily Allodiscocotylinae to include the genera *Gemmaecaputia* and *Allodiscocotyla*. Regarding the remaining four genera Tripathi included them in the subfamily Vallisiinae and transferred it to the family Allodiscocotylidae from Gastrocotylidae wherein it was assigned previously by Hargis (1957). The author agrees with Tripathi for the transfer of the subfamily Vallisiinae to the family Allodiscocotylidae but differs from him with regard to the inclusion

EXPLANATION OF TEXT-FIG. 1.

- Figs. 1-5. *Neomicrocotyle indicus* n.g. et n.sp. Fig. 1 Entire worm (ventral view), Fig. 2. Enlarged view of the gonadal region and their ducts (ventral view), Fig. 3. Terminal region of the male and female genital ducts, Fig. 4. Clamp, Fig. 5. Anchors.
Fig. 6-9. *Protomicrocotyle madrasensis* n.sp. Fig. 6. Entire worm (dorsal view), Fig. 7. Enlarged view of the gonadal region and their ducts (dorsal view), Fig. 8. Terminal region of the male and female genital ducts, Fig. 9. Clamp.

Abbreviations—

b.e.—bulbous ejaculatorius ; g.i.c.—genito-intestinal canal ; m.g.h.—male genital hooks, m.g.p.—male genital pore ; ot.—ootype ; ov.—ovary ; o.d.—oviduct ; p.h.—penis; hooks ; r.s.—receptaculum seminis ; sh.g.—shell glands ; t.—testes ; ut.—uterus ut.p.—uterine pore ; v.d.—vas deferens ; vg.—vagina ; vg.h.—vaginal hooks, vg.p.—vaginal pore ; vit.—vitellaria ; vit.d.—vitelline duct.



TEXT-FIG. 1.

of the genus *Protomicrocotyle* in the subfamily Vallisiinae for the members of the subfamily other than *Protomicrocotyle* possess clamps on both sides whereas *Protomicrocotyle* has clamps developed on one side only the clamps on the other side being suppressed. Moreover, Sproston (1946) states that the structure of the larval haptor of *Protomicrocotyle* and of the region bearing the asymmetrically placed clamps indicate this genus is clearly of different evolutionary line from *Vallisia*. Besides, complete suppression of clamps on one side as in *Monaxine* and *Urarine* formed not only the basis to differentiate them from the heteraxinids but also for the creation of a new subfamily to include them (vide Unnithan, 1957). Hence, in view of these facts, the author favours the removal of the genus *Protomicrocotyle* from the subfamily Vallisiinae and favours the revival of the subfamily Protomicrocotylinae to include the genus and it is assigned in the family Allodiscocotylidae as the third subfamily.

Genus *Bilateracotyla* was assigned as gen. inq. in the subfamily Vallisiinae by Tripathi (1959). The author favours the separation of the genus from the subfamily since it possesses microcotylid clamps quite unlike other members of the subfamily and suggests its inclusion in a separate subfamily. Subfamily Bilateracotylinae was provisionally created by Chauhan (1953) to include his genus *Bilateracotyle* since its inclusion in the subfamily Protomicrocotylinae will be misleading when, in case, the type genus *Protomicrocotyle* constituting it cannot be removed from the family Gastrocotylidae to which he has assigned. As the subfamily name Bilateracotylinae was created already, the author adopts it here for the genus *Bilateracotyle* and it is assigned provisionally under the family Allodiscocotylidae.

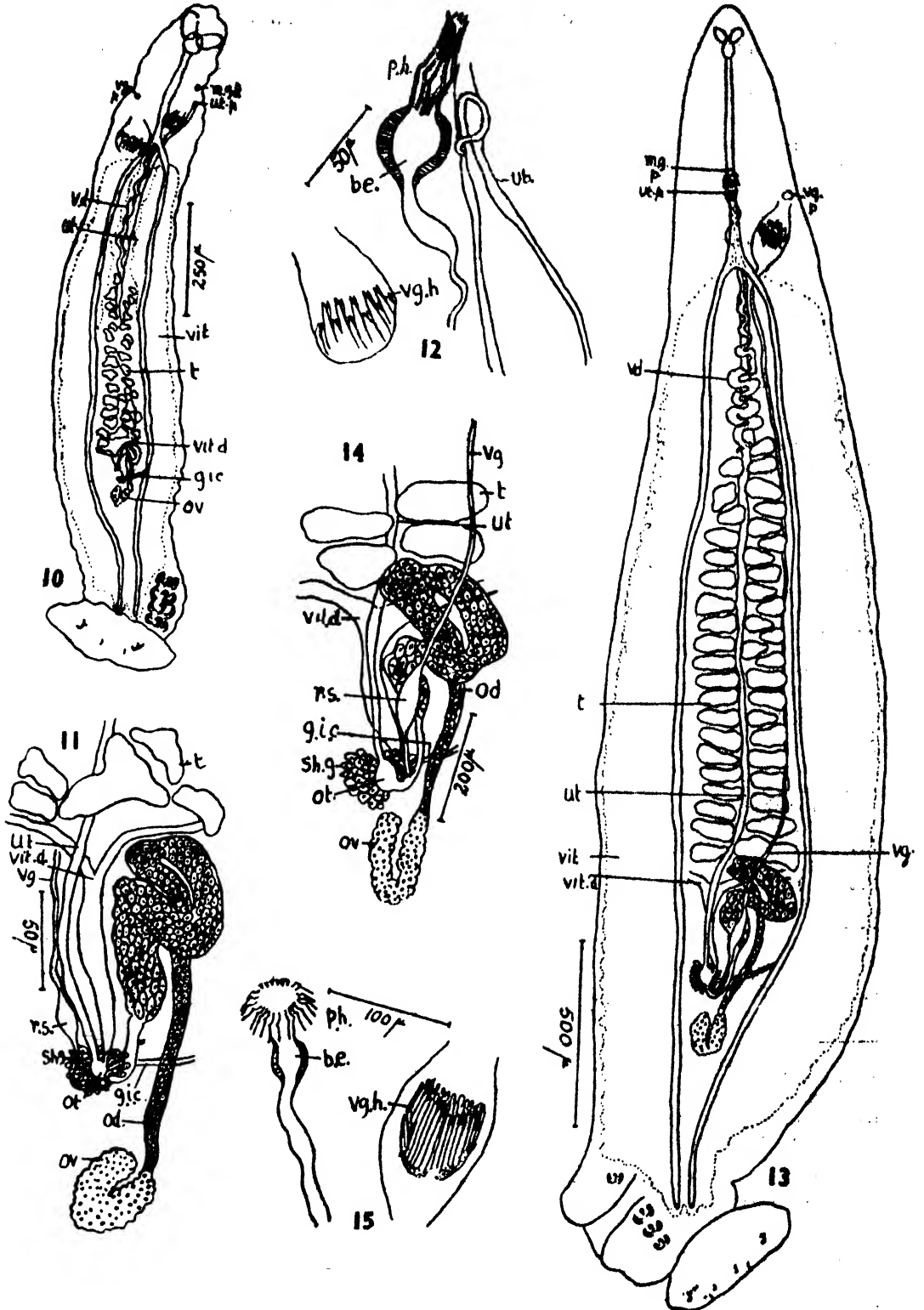
Neomicrocotyle n.g. and *Bilateracotyle* Chauhan (1945) agree with each other in the possession of pre-ovarial testes, microcotylid clamps limited to a maximum of eight but the former differs from the latter in the complete suppression of clamps on one side. Hence it was not assigned in the subfamily Bilateracotylinae. The genera *Neomicrocotyle* and *Protomicrocotyle* agree with each other in the complete suppression of clamps on one side but they differ from each other in the nature of the clamp structure and their inclusion in the same subfamily would result in its having genera with two different types of clamps. Hence the author suggests that the genus *Neomicrocotyle* is included in a separate subfamily and a new subfamily Neomicrocotylinae is created here to accommodate the genus. Its diagnosis is as follows: Allodiscocotylidae having body asymmetrical in the posterior region; with four clamps of the microcotylid type arranged in a linear row on the lobed side of the body near the hinder region; with dumb-bell shaped haptor having two pairs of anchors.

Though the possibility of creating a new family for the members of these two subfamilies (Bilateracotylinae and Neomicrocotylinae) might exist, it is not attempted at, here, since our knowledge of these forms is limited to two genera and two species and hence they are provisionally assigned in the family Allodiscocotylidae. The

EXPLANATION OF TEXT-FIG. 2.

- Figs. 10-12. *Protomicrocotyle minutum* n.sp. Fig. 10. Entire worm (dorsal view), Fig. 11. Enlarged view of the gonadal region and their ducts (dorsal view), Fig. 12. Terminal region of the male and female ducts.
Figs. 13-15. *Protomicrocotyle mannarensis* n.sp. Fig. 13. Entire worm (dorsal view); Fig. 14. Enlarged view of the gonadal region and their ducts (dorsal view); Fig. 15. Terminal region of the male and female genital ducts.

Abbreviations used are the same as before.



Text-fig. 2.

inclusion of these two subfamilies necessitates an emending of the family diagnosis and the emended diagnosis is as follows: Diclidophoroidea having the body and haptor symmetrical or both asymmetrical or body only asymmetrical; with a limited number of clamps (upto a maximum of eight) which are either of the gastrocotylid type or microcotylid type, arranged either in two lateral rows or one row of clamps suppressed in which case a maximum of four clamps only present; with a terminal lappet having 1 to 3 pairs of anchors or without a terminal lappet; testes either pre-ovarial or pre-, para- and post-ovarial; cirrus or penis either armed or unarmed and vagina either single or double. Thus the family Alloodiscocotylidae comes to comprise of five subfamilies—Alloodiscocotylinae, Vallisiinae, Protomicrocotylinae, Bilateracotylinae and Neomicrocotylinae.

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The author expresses his sincere thanks to Dr. N. K. Panikkar, F.N.I., Fisheries Development Adviser, Government of India (formerly the Chief Research Officer at the Central Marine Fisheries) for all the help and encouragement received during the course of his work at the Station. To the Ministry of Education, Government of India, he is grateful for the award of a Senior Research Scholarship.

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PHYSIOLOGICAL STUDIES ON CHILO ZONELLUS SWINH A PEST ON MAIZE CROP

I. GROWTH ON ARTIFICIAL DIETS

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Valuable informations on the food requirements of insects associated with stored plants products have been obtained during the last two decades. The data gathered and conclusions drawn have been brought together by Trager (1953) and Lipke and Fraenkel (1956) in their recent review articles. Very little is, however, known about the nutritional physiology of the forms feeding on plants probably because of their lack of response to usual investigational methods. The scattered account on the subject has recently been put together in a review by Friend (1958). One of the most worked out phytophagous insects is European corn borer, *Pyrausta nubilalis* (Hbn.) larva (Beck *et al.* 1949, Beck 1957) where nutritional studies have led to the isolation and identification of a "resistant factor" contained in corn plant, which is at least partly responsible to impart a resistance to attack by corn borer (Smismann *et al.* 1957). Such instances point out towards the applied nature of these types of investigations in the development of resistant varieties in crops. Beck's success initiated a fresh interest among entomologists world over and some important basic informations have since been obtained as a result of investigations on phytophagous lepidopterans like *Chilo simplex* (Ishii and Hirano 1957), *Prodenia eridania* (Elliott 1955) and *Pectinophora gossypiella* (Vanderzant and Reiser 1956 a,b). Auclair *et al.* (1957) showed that nature and concentration of free amino acids in the sap of varieties of peas, *Pisum sativum* plays an important role in the resistance of peas to the Pea aphid, *Acyrtosiphon pisum* (Harr.). Such types of studies have brought forth valuable informations which have led to a better understanding of insect behaviour in relation to its host plant.

The present work is a part of a project on investigations on the resistance of plants to insects in which insect involved is *Chilo zonellus* as a pest of maize and sorghum plants. The development of a suitable dietary medium of known chemical composition is a pre-requisite to any physiological studies involving insect nutrition. A number of dietary mixtures were tried and ultimately it was possible to obtain a suitable artificial diet. The present report describes a simple method of maintaining large culture of *Chilo* in laboratory and also a suitable artificial dietary medium.

METHODS

For the study of a problem of the type as the present one, it is necessary to have (i) sufficient supply of all stages of test insects in laboratory, and (ii) a technique for keeping aseptic conditions to avoid undue interference by microbial infections. After providing for these two essential requirements, attempts can be made to develop dietary mixtures which fulfil nutritive and feeding requirements nearly as efficiently as the natural food. In this attempt various factors had to be taken into account by way of the chemical composition and physical state of food on one side and feeding habits of larva on the other.

A supply of newly hatched larvae employed in testing the suitability of artificial diets was obtained from moths reared from larvae initially collected from the field during active season which is between March to early October in Delhi. The moths readily laid eggs in small jars of 6" diameter and 8" high, covered all round internally by waxed or tissue paper, and containing cotton-wool wick soaked in sugar solution for moths to feed. The jars were covered with fine muslin and kept at a temperature of 30°C and 40-80 per cent R.H. Scale like eggs were laid in masses in overlapping pattern on the paper. An easy way to handle eggs for subsequent treatments was to cut away the mass along with the paper. These were then incubated at 30°C and 70 per cent R.H. Sometimes the eggs were treated with a sterilizing fluid but later on it was not considered necessary. The newly emerged larvae were picked up with a fine camel-hair brush and transferred to food vials. Surplus larvae were used for continuing the stock cultures maintained in laboratory on maize or jwar stems.

STOCK CULTURE

Earlier larval instars were fed on tender apical leafy part of stem while older instars could be given even pithy stem. The food was changed at least on alternate days or earlier if signs of deterioration begin showing. Initially 8" piece of stem was enough for 10 larvae but later on not more than one or two larvae per piece should be kept to avoid over-crowding which induces cannibalism.

To facilitate establishment of older larvae on food a small cavity was made at one end of the stem and a single larva was placed inside it. This was necessary for larval-feeding, everytime new food was offered, otherwise many larvae failed to bore in, wandered about and died. The larvae either pupated outside or in the food tunnel. In both the cases it was possible to collect pupae and remove them for moths to hatch.

This method worked very satisfactorily and large number of insects could be bred under laboratory conditions. At the onset of winter, however, *Chilo* population markedly decreased both in the field and the laboratory. The insect seemed to undergo a state of diapause in larval stage. In the laboratory, larval period was very much extended and a very few moths could be obtained. Majority of insects remained as larvae even under normal temperature of 30°C and with ample supply of food. This resulted in slackness in the progress of work during winter months when strength of culture remained at a very low ebb.

PREPARATION OF MEDIA

Table I shows a list of different dietary formulations together with proportions of their ingredients which were offered to *Chilo zonellus* larvae.

The dry ingredients were thoroughly ground and mixed together in a mortar and pestle. Cholesterol was dissolved in any one of the volatile solvents and added to the mixture to ensure its uniform distribution in the diet. The mixture was then added to a 250 ml. flask containing required amount of water. Carboxymethyl cellulose, when used, was first dissolved in warm water and added to the aqueous suspension. The final diet was then autoclaved at 15 lb. pressure for 20 minutes in a "Presto" pressure cooker. The sterilized material was transferred aseptically into 1" x 2" glass vials with cotton wool plug. These were allowed to stand at room temperature for 1 or 2 days until excess moisture adhering to side walls evaporated. The vials were then ready to receive the test insects.

All experiments were conducted at 30°C. In earlier trials five newly hatched were transferred to each vial containing the medium but later on one larva per



FIG. 1.

A larva of *Chilo zonellus* developing on artificial diet. The insect was removed from feeding vial to facilitate photography.

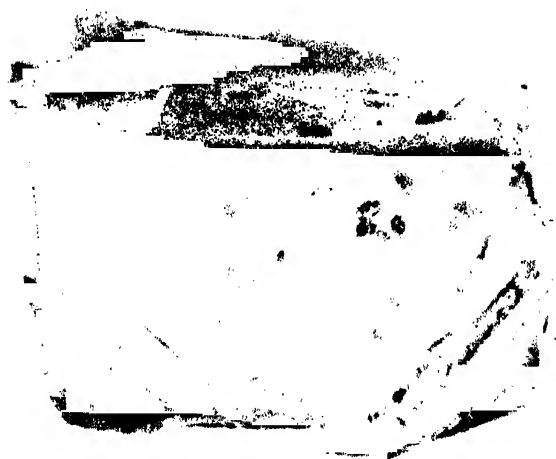


FIG. 2.

Pupa of *Chilo zonellus* developed on artificial diet. The diet and insect were removed from feeding vial to facilitate photography.

tube was used to avoid cannibalism. Before putting the insect in the tube it was found necessary to create a very narrow gap all round the food so that the larva may be placed in such a manner that its ventral surface was in contact with food while the dorsal touched the glass surface. This fulfilled one of the very important physical conditions for larval establishment by providing stimulus essential for initiating establishment and continued feeding. Back and Stauffer's method (1950) of offering food as "feeding stations" met with little success with *Chilo*. The feeding vials were covered with black paper to exclude the light for photo-negative larvae.

TABLE I

Showing composition and general performance of various artificial dietary media used for growing larvae of Chilo zonellus.

Ingredients	Diet I Gm.	Diet II Gm.	Diet III Gm.	Diet IV Gm.
Casein	2.70	5.47	2.73	5.50
Glucose	2.70	5.47	2.73	5.50
Salt mixture	0.20	0.44	0.22	0.44
Yeast	1.10	2.20	1.10	2.20
Choline chloride	0.40	0.09	0.40	0.09
Cholesterol	0.10	0.22	0.11	0.22
Cellulose source	5.00	5.00	2.50	2.50
	(Filter paper)	(Filter paper)	(C.M.C)	(C.M.C)
'Leaf factor'	Aqueous extract +	Ether extract +	—	1.80 (Dry leaves)
Agar	1.60	3.75	1.67	3.50
'Butoben'*	—	0.20	0.20	0.10
Water	62.5	125.0	62.50	125.00
General performance	Inadequate	Inadequate	Inadequate but partially adequate for 3rd stage larvae	Adequate for all stages

C.M.C. = Carboxymethyl cellulose

+ = Extracted from 14 gm. of fresh leaves.

* = Methyl para-oxybenzoate.

RESULTS AND DISCUSSION

Performance of media

The diets were offered in the manner described above. Observations on growth and metamorphosis were regularly recorded. The diets were changed as soon as visual signs of deterioration by way of contamination were observed. Diet I (Table I) failed to support growth of young larvae which died within few days but older individuals could subsist for more than 20 days without pupating. Similarly diets II and III also proved inadequate to newly hatched larvae. These diets were lacking in a suitable stabilizing agent or 'leaf factor' which later proved necessary for imparting a desired property to media. Filter paper, used as a stabilizer, could never be mixed uniformly with other dietary ingredients but when carboxymethyl cellulose was procured and substituted for the paper pulp, larval response improved to a great extent. The diets were further improved by the addition of dry-leaf powder instead of aqueous or ether extracts of leaf. Medium IV

which contained both carboxymethyl cellulose and dry leaves proved the best semi-artificial medium in which newly hatched larvae of *Chilo zonellus* could establish, feed and pupate to give rise to normal moth which oviposited viable eggs. The average developmental period up to adult stage was 39 days. Plate XXII, Figs. I and II show a larva and a pupa developed on this synthetic diet.

Chilo zonellus larvae responded favourably only to one of the artificial media (IV) which contained dry leaf and offered the necessary physical texture and chemical nutrients necessary for larval establishment and growth. The presence of small fraction of dry 'maize-leaf' was vital to at least young larvae which died very early if the diets were deficient in this respect. The indispensibility of leaf factor is a phenomenon which has also been observed in a few other phytophagous insects like *Chilo simplex* (Ishii 1952), *Pyrausta nubilalis* (Beck, 1953) but not in *Pectinophora gossypiella* (Vanderzant 1956 a,b). The fact that a tissue borer like *Chilo* is most vulnerable in its first larval stage, makes it imperative to investigate fully the implication and scope of this phenomenon of "leaf-factor"-deficiency which could perhaps be exploited for the isolation of factors or characters for which varieties may be bred so as to evolve strains resistant to insect attack. Such an aim is extremely desirable and calls for sustained efforts to achieve the objective.

A knowledge of the exact chemical nature and biological activity of 'Leaf factor' will be of great value to entomologists and plant breeders. Preliminary observations made on the orientation of newly hatched larvae in relation to plant indicate that they tend to migrate towards apical leafy portions in the growing region of plant and feed on leaves for a few days before boring into the stem. Larvae which were denied an opportunity to feed on leaves tend to become very weak and fail to bore in. It may, however, be borne in mind that a large number of larvae in nature die owing to various causes other than the chemical nature of host plant and this fact must not be over-looked while interpreting the larval orientation in relation to its establishment in host plant.

The diet evolved is only semi-synthetic as it still contains at least two materials of organic origin—'leaf factors' and yeast. Replacement of these two ingredients by suitable substances of known composition is necessary before the exact role of individual ingredients can be ascertained.

Indispensibility of dry leaves even in a yeast diet shows that the nutrition of *Chilo* like some other phytophagous larvae may differ from large number of insects (Lipke and Fraenkel 1956) where yeast provides almost all the accessory growth factors required to supplement a synthetic diet.

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IMPORTANCE OF SOME TAXONOMIC CHARACTERS IN THE FAMILY PHYTOSEIIDAE BERL., 1916, (PREDATORY MITES) WITH NEW RECORDS AND DESCRIPTIONS OF SPECIES

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ABSTRACT

The paper deals with the importance of some characters, the number, the arrangement, the nature, the position and the relative length of setae together with some anatomical characters in the taxonomy of mites.

Notes on six species of mites belonging to family Phytoseiidae not recorded in the Indian Union before have been given. Three new species of predatory mites belonging to the family Phytoseiidae have also been described.

For more than five decades, many species of the family Phytoseiidae have attracted the attention of economic entomologists all over the world for the biological control of Phytophagous mites infesting agricultural and horticultural crops. It must, however, be emphasized that this early interest was purely of an academic nature. Only within recent years, these beneficial mites have attracted the attention of entomologists all over the world. In the main, the reason for this has been the destruction of predatory species that maintained the population of phytophagous mites in a condition of equilibrium by the widespread use of organic insecticides especially, chlorinated hydrocarbons, over very large areas of agricultural tracts. As Thompson (1930) observes, "Since organisms increase in geometrical progression, the survival in each generation of even two or three females above the number necessary to maintain the population at an even level, may produce in a relatively short time disastrous results." So in this context, the role of predatory mites assumes particular significance.

With the recognition by economic entomologists of the importance of these predaceous species in the population balance in nature, the taxonomy of this family has received considerable attention and has been critically discussed and studied in a number of recent contributions and no where more ably than by Nesbitt (1951) in his excellent review of the European species, including the many previously described by Oudemans (1929). The basis of specific separation adopted by him was based on that devised by Garman (1948) and this has since been widely used and referred to by other workers in America and England. It is mainly concerned with the setation of the dorsal and ventral surfaces.

As the first reviewer of the group, Nesbitt (1951) had to exercise a certain amount of choice in the fixing of genotypes and hence genera. Great care was taken because he realised that in doing so he was "adopting a somewhat arbitrary taxonomic position". As he himself observes, "It is my belief, however, that the exigencies of the situation warranted such an action and that is preferable to maintain taxonomic names, which in the past have expressed a true biological relationship than to discard them, because the type specimens on which they are based are no longer available".

For a long time, the taxonomy of the species of Phytoseiinae remained in a state of confusion, as very few older type specimens were available for study and the characters on which the descriptions were based were so insufficient and vague that it was impossible to come to any definite conclusion based on proper identification.

Classification in Acarina is chiefly based upon the chaetotaxy and the number and position of stigmatal openings. The family Phytoseiidae can be readily separated from all other families of Mesostigmata in having less than twenty pairs of setae on the dorsal shield in the proto-, deuto-nymphal and adult stages. The genera comprising this family are separated by the chaetotaxy of the dorsal shield. But here again some confusion has arisen as various workers (Nesbitt 1951, Womersly, Evans, Bernhard and Chant) have taken different aspects of setal characters into consideration and we have yet to decide which one is more rational and reliable and least open to errors of observation and misinterpretation, namely, number, arrangement, nature, position or relative length.

Now we shall take up these characters one by one and discuss their importance in the classification of Phytoseiinae.

1. Number of setae

Number of setae is a character, which helps not only to determine a particular species but also its genus and family. The gross number determines the family when we say that Phytoseiids are characterized by having less than 20 pairs of setae on the dorsal shield; with not more than 18 pairs of dorsal setae in the genus *Typhlodromus* Sch. and likewise some number is fixed for other genera too. But again, the number may vary within the genus and this helps us in determining a species. Thus we see that this character need not be necessarily fixed but may vary within certain limits. This variation within limits should be allowed and it is unwise to have restriction as to its being very specific. We have observed during the course of our studies that the number of setae on the female ventri-anal plate may vary within a species but the sum-total of all that are present on and surrounding the shield always remains constant. This is what we have seen in the case of *Phytoseius macropilis* (Banks) and *Phytoseius minutus* sp.n. Chant (1957) observed this variation in the progeny of a single female and thus ascribed it to intraspecific variation.

2. Arrangement of setae

The arrangement of setae on the dorsal shield is an important character. The number of rows of setae i.e., lateral, dorsal and median, on the dorsum is fixed within the family and thus is of great familial value.

3. Nature of setae

This is a character of generic value only. Setae may be simple or slightly serrated as in *Typhlodromus* or distinctly serrated and thickened as in *Phytoseius* or thick and thorn-like as in *Seiulus*.

4. Position of setae

Position of setae is of generic as well as of specific value. The scapular seta (S_1) is on the dorsal shield in both the adult forms of *Phytoseius* Ribaga, whereas it is on the interscutal membrane in the females of *Typhlodromus* Sch. Thus the two genera can be readily separated on the basis of the position of S_1 .

The position of M_1 with respect to L_7 and L_8 and the position of the setae on the ventri-anal plate may vary within a genus and thus help in an easy determination of a species.

5. Relative length of setae

This is a character of only specific value. It is a relative term and should not be relied upon for any generic or subgeneric determinations, as some workers have done it. The various genera comprising Phytoseiinae can be separated on

the basis of nature, position and number of the dorsal setae regardless of their relative lengths. In *Amblyseius* as described by Berlese (1914), L_1 , L_2 and M_2 are longer than other dorsal setae, whereas in *Typhlodromus* as defined by Nesbitt (1951) they are not. However, in *Typhlodromus* all dorsal setae may be of extreme length, as in *T. longipilus* Nesbitt and *T. occidentalis* Nesb., and this clearly shows that length is not a character of generic value but is the most valid criteria for specific determinations.

3. Some anatomical and other minor characters

There are some anatomical and other minor characters which may help in an easy determination of a species. These are the shape of spermatophoral process in males, shape of coxal glands, number of macrosetae on leg IV and the number of dorsal pores. Out of these, the shape of "coxal glands" alone may afford a good difference between the two closely allied species. This is what has been observed in the case of *T. (A.) marinus* (Willman) and *T. (A.) delhiensis* sp. nov. (Narayanan and Kaur, 1960). These two species are very closely related except for the shape of their "coxal glands" which clearly separate the two. Unfortunately, this character has not been given the importance it deserves and there is almost no mention of this interesting structure in most of the older descriptions. These structures, though internal, can be clearly seen when properly cleared and mounted. In our opinion, these characters should be thoroughly examined so as to bring out the morphological differences between the closely related species.

The recent publication of Chant (1958) deserves special mention as he has brought in his study a wider sweep of taxonomic characters based on the morphological studies of immature stages. This will lead to a most satisfactory, rational and natural system of classification. As Evans (1955) states, "Our present conception of familial and generic divisions is, however, most unsatisfactory, but investigations on the developmental stages may result in a more natural classification than at present in use."

The basis for all applied research is the taxonomy of the group concerned, even as the basis of the successful introduction and establishment of a parasite or predator, to control a particular pest is a thorough knowledge of the biology and ecology of the species.

Practically no work has been carried out so far in India on the taxonomy of this interesting group of predatory mites. In this paper some new records and descriptions of species belonging to Phytoseiidae and Cheyletidae are given. The species now recorded are refigured from Indian material.

Family Phytoseiidae

Genus *Phytoseius* Ribaga

Phytoseius macropilis (Banks, 1909) Type species

(Text-fig. 1, Fig. 2a-d)

Nesbitt, 1951, *Zool. Verh. Leiden* 12.

Cunliffe and Baker, 1953, *Pinellas Biol. Lab. Pub.*, I.

Womersley, 1954, *Aust. J. Zool.*, Vol. 2.

Chant, 1958, *J. Linn. Soc. Lond. Zool.*, XLIII (294).

Chant, 1959, *Canad. Ent. Supplement* 12, XCI, 5-165.

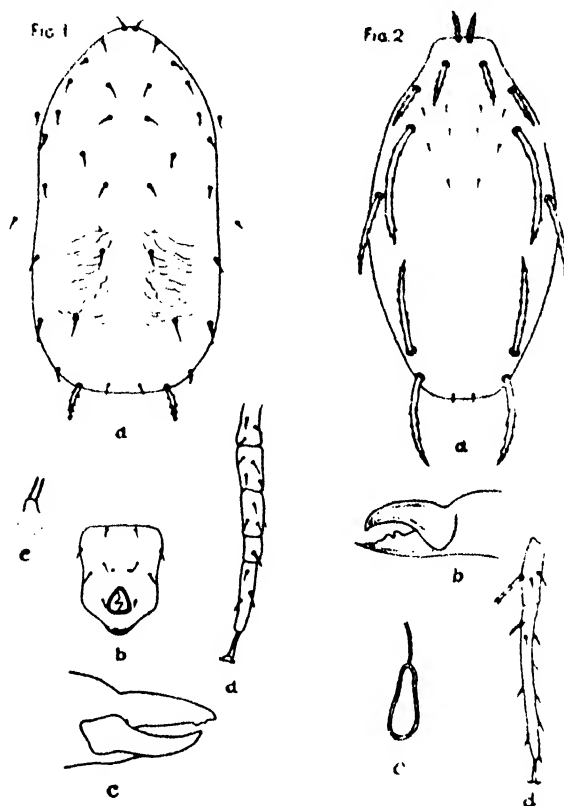
Host :—Tetranychid mites

Host plants :—Leaves of Fig, Mulberry, Compositae (unidentified) and *Lantana camara*.

Loc :—New Delhi.

Coll :—R. B. Kaur.

This is a very common species found almost all through the year on one or the other above mentioned host plants. It is very wide in its distribution and has been recorded from almost all the European countries as a predator of Tetranychidae and Eriophyidae on leaves of *Salix*, fig and grapevine.



TEXT-FIG. 1.

Fig. 1. *Typhlodromus* (*Typhlodromus*) *bakeri* (a) Adult female (dorsal view, $6.3\times40\times$); (b) Ventri-anal plate Female ($6.3\times40\times$); (c) Chelicera female ($10\times100\times$); (d) IV leg ($6.3\times40\times$); (e) Coxal gland ($10\times100\times$).

Fig. 2. *Phytoseius macropilis* (a) Adult female (dorsal view, $6.3\times40\times$); (b) Chelicera female ($10\times100\times$); (c) Coxal gland ($10\times100\times$); (d) leg ($6.3\times40\times$).

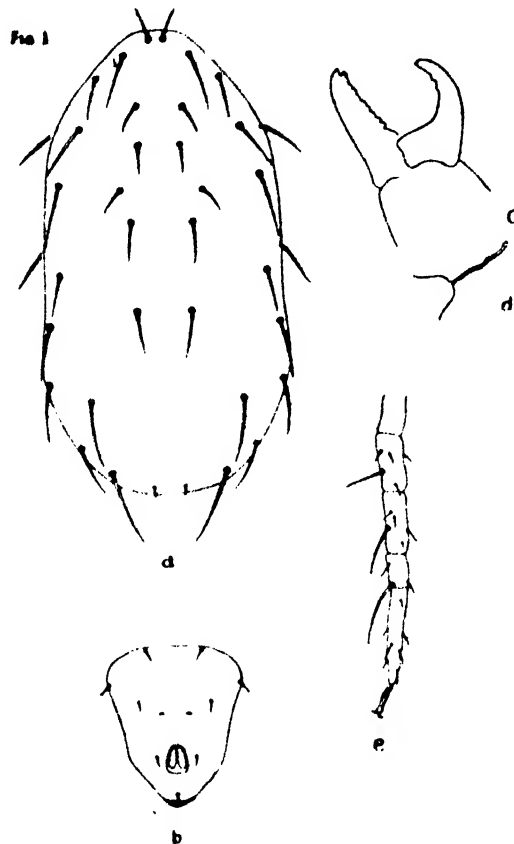
As to its identification, a careful examination reveals that there are only two macrosetae on leg IV, on tibia and basitarsus. Of these two, the tibial is longer and blunt (Text-fig. 1, Fig. 2d). Chant (1957) while comparing this species with *P. plumifer*, mentions that there are three macrosetae on leg IV. Nesbitt (1951) shows three in his drawing of the female and two in the male, whereas we have observed only two in both the sexes. Hence, we feel that this may also be an intraspecific character like the number of setae on the ventri-anal plate of female.

Typhlodromus (*Amblyseius*) *fallacis* (Garman)
(Text-fig 2, Fig. 1a-c)

Garman, 1948, *Connecticut Agric. Expt. Sta. Bull.*, 520 : 13.
Nesbitt, 1951, *Zool. Verh. Leiden*, 12.

Cuniffe and Baker, 1952, *Pinellas Biol. Lab. Pub.* No. 1:3.

Womersely, 1954, *Aust. J. Zool.*, Vol. 2, (174).



TEXT-FIG. 2.

Fig. 1. *Typhlodromus (Amblyseius) fullacia* (a) Adult female (dorsal view, $6.3 \times 40 \times$). (b) Ventri-anal plate female ($6.3 \times 40 \times$); (c) Chelicera female ($10 \times 100 \times$); (d) Coxal gland ($10 \times 100 \times$); (e) IV leg ($6.3 \times 40 \times$).

Collected by means of Berlese funnel from seeds of "Sowank" (*Echinichloa-Crusgalli*).

Loc :—Karnal.

Coll :—Roshan Lal, 4-9-1959.

This species has been recorded in N.S.W. from "Thrips infested banana"; in Connecticut, U.S.A., from apple leaves and in Canada from a number of orchard trees as feeding on Tetranychids.

Typhlodromus (Amblyseius) ovalis Evans
(Text-fig. 3, Fig. 1a-f)

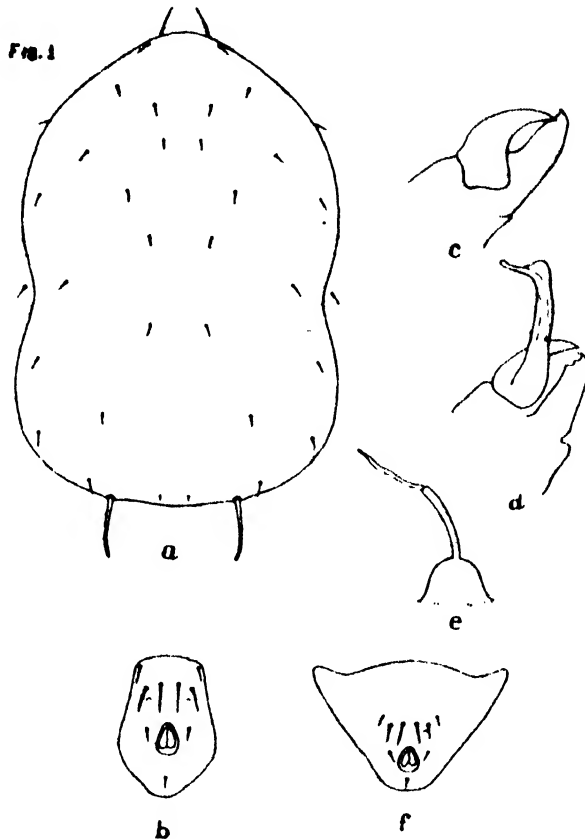
Evans, 1953, *Ann. Mag. Nat. Hist.* (12), VI, 449.

This species has so far been recorded only from Malaya as feeding on Tetranychids on rubber at Kuala. In our country, it appears to be a common species

and has been collected from the leaves of *Ficus* sp., *Terminalia arjuna* (both infested with Tetranychid mites) and Banana in Bombay and from an unidentified plant in Mysore.

Loc :—Aarey Milk Colony, Bombay.

Coll :—R. B. Kaur, 5-12-1959.



TEXT-FIG. 3.

Fig. 1. *Typhlodromus* (*Amblyseius*) *ovalis* (a) Adult female (dorsal view, $6.3 \times 40 \times$); (b) Ventri-anal plate female ($6.3 \times 40 \times$); (c) Chelicera female ($10 \times 100 \times$); (d) Chelicera male ($10 \times 100 \times$); (e) Coxal gland ($10 \times 100 \times$); (f) Ventri-anal plate male ($6.3 \times 40 \times$).

Typhlodromus (*Amblyseius*) *asiaticus* Evans

Evans, 1953, *Ann. Mag. Nat. Hist* (12), VI.

On leaves of arecanut seedlings, Mysore.

This species has so far been recorded only from Malaya and Indonesia from some unidentified leaves.

Typhlodromus (*Typhlodromus*) *bakeri* (Garman)
(Text-fig. 1, Fig. 1a-e)

Garman, 1948, *Bull. Conn. Agric. Exp. Sta.*, 520 : 15.

Nesbitt, 1951, *Zool. Verh. Leiden*, 12 : 36.

Cunliffe & Baker, 1953, *Pinellas Biol. Lab. Pub.*, 1 : 10.

Womersley, 1954, *Aust. J. Zool.* 2 (169-191).

Chant, 1956, *Canad. Ent.* 87 (496-503).

Chant, 1958, *J. Linn. Soc. Lond. Zool.* XLIII, No. 295 (599-643).

Female : Dorsum rugose of .273 mm. in length and 0.15 mm. in breadth bearing 18 pairs of setae, in 10 in the lateral, 6 in the dorsal and 2 in the median rows. Posterior lateral setae distinctly and M_2 slightly serrated. Ventrianal shield with 4 pairs of pre-anal setae and without any creases or folds encircling the anus as has been mentioned in all the previous descriptions. Fixed digit of chelicera with 5 teeth and pilus dentilis and movable with 3 minute teeth. Leg IV with only one short and blunt macroseta on basitarsus. Peritreme almost meeting medially anteriorly.

On leaves of Grapevine.

Loc :—I.A.R.I., New Delhi.

Coll :—R. B. Kaur, 25-8-1959.

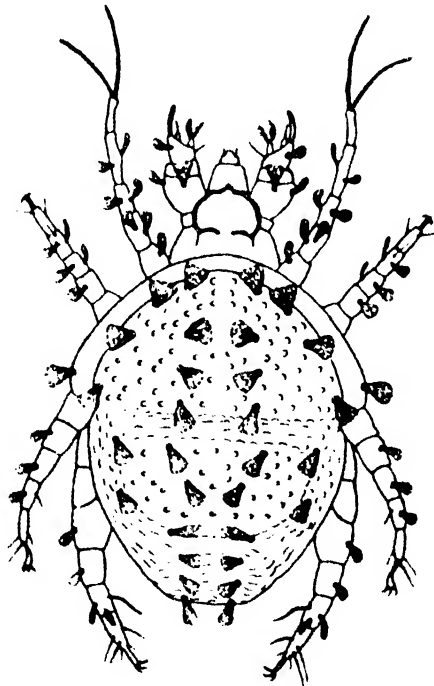
The species has been collected from apple bark in U.S.A. and Canada from "Twigs of Peach bearing eggs and Green Peach Aphis" and from "Pear twigs", N.S.W. In England it has been recorded by Chant from the bark of a number of orchard trees in Kent and Essex.

Family Cheyletidae
Genus *Cheletogenes* Oudemans
Cheletogenes ornatus (Can. & Fan.)

Oudemans, 1905, *Ent. Ber. Nederl. Ver.*, Vol. I (21), p. 208.

Womersley, 1942, *Trans. Roy. Soc. South Aust.*, Vol. 66(1) : 85.

FIG. 1



TEXT-FIG. 4.

Cheletogenes ornatus (a) Adult female (dorsal view, 6.3x × 40x).

Baker, 1949, *Proc. U. S. Nat. Mus.*, Vol. 99, No. 3238, pp. 268-320.

Host :—Tetranychid mites.

Host Plant :—Leaves of fig.

Habitat :—New Delhi.

Coll :—R. B. Kaur, 10-7-1959.

It is a small, yellow species with squamiform serrate dorsal, formal and genual setae (Text-fig. 4).

During the rainy season i.e., in the months of July and August, it is commonly found on the leaves of fig. It has been recorded from California in lemon buds with *Aceria sheldoni* (Ewing). It has also been recorded from Italy, China, Hawaiian Islands, West Indies and Australia, associated with scale insects or with eriophid mites on which it preys.

Phytoseius minutus sp. nov.

(Text-fig. 5, Fig. a-h)

Female : Length 0.26 mm.; breadth 0.14 mm.; dorsum rugose, shield single with 16 pairs of setae (including S_1 , which is associated with I_4 and I_6), arranged in a lateral row of seven, a dorsal of six and a median of two pairs (Text-fig. 5, Fig. 1a). S_1 on the dorsal shield, S_2 present. All lateral setae (except I_2 and I_4), D_1 , S_1 and M_2 thickened and distinctly serrated; and all dorsal setae (except D_1) M_1 , S_2 , I_2 and I_4 short and smooth. The relative length of these setae is as below :

(I_1 - I_7) 19 : 4 : 15 : 4 : 28 : 25 : 22 : (D_1 - D_6) 8 : 15 : 1 : 1 : 1.5 : 1.5

(M_1 - M_2) 1 : 21 : (S_1 - S_2) 14 : 4.

There are four pairs of dorsal pores as shown in Text-fig. 5, Fig. 1a, of which the 2nd pair is well-developed and covers the setae M_1 almost completely.

Peritreme with its stigmata, opening between coxae III and IV and anteriorly extending on to dorsum and almost meeting in the mid-line.

Ventrally, body covered by the usual shields. Ventri-anal plate longer than broad, 0.085 mm. in length and 0.065 mm. in width; shaped as in figure (Text-fig. 5, Fig. 1b), with 3 pairs of setae in addition to para-and post-anals. Parapodal plates absent.

Gnathosoma and maxillary palps normal for the group. Fixed digit of chelicera with two prominent and well separated teeth; movable digit with one weak tooth (Text-fig. 5, Fig. 1c).

Coxal glands as figured (8). (Text-fig. 5, Fig. 1d).

Leg IV with three macrosetae, on genu, tibia and basitarsus. Setae blunt (Text-fig. 5, Fig. 1e).

Male : Length .12 mm., width .12 mm. Dorsal chaetotaxy resembling that of female, relative lengths of the dorsal setae are the same, though they are smaller comparatively (Text-fig. 5, Fig. 1f).

Ventri-anal shield with three pairs of setae in addition to para and post-anals, arranged as in *P. macropilis* (Banks) (Text-fig. 5, Fig. 1g).

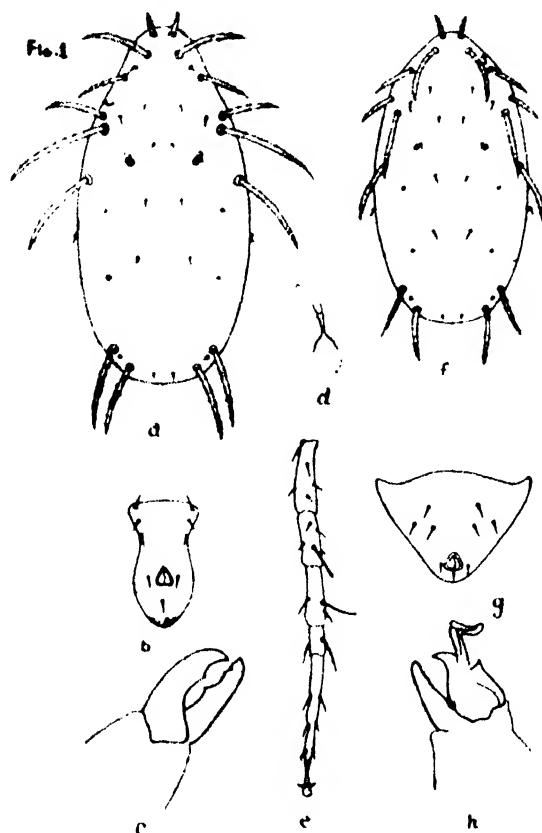
Fixed digit of chelicera with three teeth; movable digit with one tooth and a hammer-shaped spermatophoral process. (Text-fig. 5, Fig. 1h).

This species may be distinguished from *P. plumifer* by the presence of three macrosetae on leg IV and by the absence of para-podal plates. It is also very close to *P. nahuatlensis* DeLeon from which it differs in the relative length of setae on the dorsal shield.

Described from 7 females and 5 males, collected from Tetranychid infested leaves of *Hibiscus esculentus* H. at Indian Agricultural Research Institute, New Delhi.

Holo-, allo- and para-types deposited in National Pusa Collection, I.A.R.I., New Delhi.

Coll.: R. B. Kaur, 7th March, 1959.



TEXT-FIG. 5.

Fig. 1. *Phytoseius minutus* sp. nov. (a) Adult female (dorsal view, $6.3 \times 40 \times$); (b) Ventrianal plate female ($6.3 \times 40 \times$); (c) Chelicera female ($10 \times 100 \times$); (d) Coxal gland ($10 \times 100 \times$); (e) IV leg ($6.3 \times 40 \times$); (f) adult male (dorsal view, $6.3 \times 40 \times$); (g) Ventri-anal plate male ($6.3 \times 40 \times$); (h) Chelicera male ($10 \times 100 \times$).

Typhlodromus (Typhlodromus) confusus, new species
(Text-fig. 6, Fig. 1a-d)

Female: Dorsal shield faintly reticulated, .273 mm. in length and .15 mm. in breadth; with 17 pairs of setae, nine in the lateral, six in the dorsal and two in the median rows. (Text-fig. 6, Fig. 1a). All setae smooth except M_2 and L_9 which are slightly serrated. Relative length of the setae as follows:

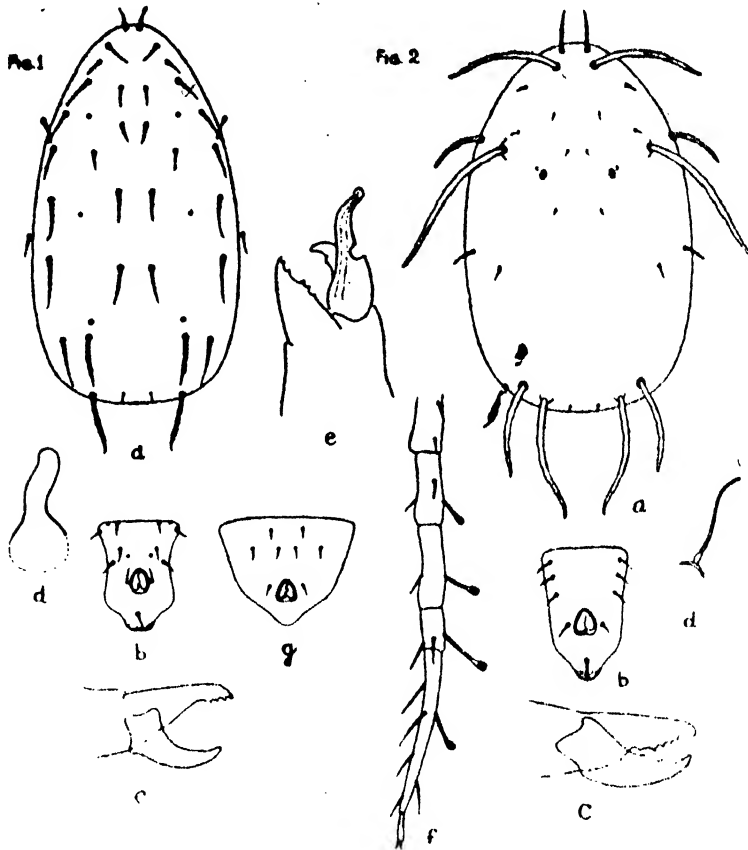
(L_1 L_9) 7 : 5 : 7 : 8 : 9 : 12 : 13 : 13 : 13 : (D_1 - D_6) 5 : 5 : 5 : 8 : 10 : 2,
(M_1 M_2) 5 : 14.

Except D_6 , all other setae in the posterior half of the dorsal shield are comparatively longer than those in the anterior half. Three pairs of pores present on dorsum as shown in Text-fig. 6, Fig. 1a.

Setae S_1 and S_2 on interseutal membrane. Peritreme almost meeting in the mid-line anteriorly. Sternal shield normal. Ventri-anal shield longer than broad,

length .085 mm., breadth .052 mm. with 4 pairs of pre-anal setae and a pair of pores. Parapodal plates present, one pair long and narrow, the other minute.

Coxal glands with thick and short duct (Text-fig. 6, Fig. 1d). Gnathosoma and maxillary palps normal. Movable digit of chelicera toothless, fixed with three minute teeth. Pilus dentilis not seen. Leg IV without any macrosetae.



TEXT-FIG. 6.

Fig. 1. *Typhlodromus (Typhlodromus) confusus* sp. nov. (a) Adult female (dorsal view, $6.3 \times 40 \times$); (b) Ventri-anal plate female ($6.3 \times 40 \times$); (c) Chelicera female ($10 \times 100 \times$); (d) Coxal gland ($10 \times 100 \times$).

Fig. 2. *Typhlodromus (Amblyseius) orientalis* sp. nov. (a) Adult female (dorsal view, $6.3 \times 40 \times$); (b) Ventri-anal plate female, ($6.3 \times 40 \times$); (c) Chelicera female ($10 \times 100 \times$); (d) Coxal gland ($10 \times 100 \times$); (e) Chelicera male ($10 \times 100 \times$); (f) IV leg ($6.3 \times 40 \times$); (g) Ventri-anal plate male ($6.3 \times 40 \times$).

This species comes closer to *T.(T.) tilus* with which it was confused in the beginning and hence the name *confusus* but differs from it chiefly in the length of setae and dentition of the chelicera.

Male: Unknown.

Described from a single female specimen collected from the leaves of sunflower, Delhi.

Holo-type deposited in the National Pusa Collection, I.A.R.I., New Delhi. Coll.:—R. B. Kaur, 14-7-1959.

Typhlodromus (Amblyseius) orientalis, new species
(Text-fig. 6, Fig. 2s-g)

Female : Dorsal shield .3 mm. in length and .178 mm. in breadth ; with thirteen pairs of setae, five in the dorsal, six in the lateral and two in the median rows. (Text-fig. 6, Fig. 2a). S_1 and S_2 on interseutal membrane. Setae L_1 , L_4 , L_6 and D_1 and M_2 thickened and slightly serrated (as in *Phytoseius* Ribaga) and measure .197 mm., .143 mm., .118 mm., .035 mm. and .078 mm. in length respectively. The rest of the setae are simple and minute. A pair of prominent dorsal pores situated in close proximity to setae M_1 as shown in Text-fig. 6, Fig. 2a.

Peritreme with stigmata, opening between coxae III & IV and anteriorly extending on to dorsum beyond coxae I.

Ventrianal plate longer than broad, shaped as in Text-fig. 6, Fig. 2b with three pairs of setae in addition to para-anals and post-anals.

Gnathosoma and maxillary palps normal. Fixed digit of chelicera with a row of about six teeth and movable with two (Text-fig. 6, Fig. 2c). "Coxal glands" as figured. (Text-fig. 6, Fig. 2d).

Leg IV with four macrosetae, on genu, tibia, basitarsus and tarsus. Setae spatulate (Text-fig. 6, Fig. 2f).

Male : Length .208 mm., breadth .13 mm.; chaetotactic pattern similar to that of female.

Ventrianal shield with three pairs of setae arranged as in Text-fig. 6, Fig. 2g. Fixed digit of chelicera with 5 teeth, movable with one and spur shaped spermatophoral process (Text-fig. 6, Fig. 2e).

Described from 6 females and 2 males. Collected from leaves of *Ipomoea* and cotton.

Loc. : Chembur, Bombay.

Coll. : R. B. Kaur, 3-12-1959.

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*Original not seen.

†It refers to the date of issue of separates of this work and is therefore the date of publication of the new genus.

OBSERVATIONS ON THE EMBRYONIC AND LARVAL DEVELOPMENT OF SOME ESTUARINE PALAEMONID PRAWNS

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ABSTRACT

An account of the embryonic development, hatching and the structure of the early larvae of *P. malcomsonii*, *P. rudis*, *P. scabriculus*, and *P. mirabilis* is given. A comparison between the different species described, shows certain differences in size, time taken for development and hatching and the time of appearance or functioning of various embryonic structures. Differences in the nature of chromatophores and their arrangement in the 1st stage larvae of all the species studied, have been described and the salient points of variation in the morphometric characters that distinguish the larvae of different species are discussed. The 2nd stage larva of *P. mirabilis* is described in detail.

Prawns belonging to the genera *Palaemon* and *Leander* form an important fishery in the Hooghly estuary, accounting for about 70 per cent of the total catches of prawns in this region. During the course of studies on the populations of estuarine prawns, it was found necessary to assess the fluctuations in the abundance of juveniles during the various seasons in the year, hence this study on early embryonic and larval history was started with a view to establishing the identifying characters of eggs and larvae of different species. Nataraj (1947) has described in some detail the early development of *Palaemon idae* Heller, and Aiyer (1949), its embryology. Das (1935) and John (1947) reported having studied the development stages in *Palaemon lamarrei* H.M. Edw., and *Palaemon carcinus* Fabr., respectively, but the detailed descriptions have not yet been published. Menon (1938) has given the first detailed descriptions of the first two larval stages in *Palaemon carcinus* and *Palaemon rudis*. But for these, there are no published reports on the developmental features of any other species of the genus *Palaemon* of Indian waters.

This paper deals with four of the more important species of prawns, namely, *Palaemon malcomsonii* H.M. Edw., *P. rudis* Heller., *P. mirabilis* Kemp., and *P. scabriculus* Heller.

P. malcomsonii was recorded as occurring in the various parts of South India (Henderson and Matthai, 1910); as common in "peninsular rivers that drain into the Bay of Bengal; Malabar and east coast of India upto the Mahanadi delta; West coast of India from Indus delta to the northern limit of Malabar coast." (Tiwari, 1955). The species occurs in the Middle and upper zones of the river Hooghly and closely resembles a new variety described by Schenkel (1902) under the name *Palaemon spinipes* Var. *birmanicus*. However, in identifying this species as *P. malcomsonii*, the author has followed Holthuis (1950) who has observed, "The specimens of this new variety show all characters mentioned and figured by Henderson and Matthai for *M. malcomsonii*, so that I see no reason whatever to separate the two forms."

P. rudis was recorded as occurring in "Madras and Kakinada" in South India (Henderson and Matthai, 1910) from "Malabar and east coast of India upto Mahanadi area and the deltaic Bengals" (Tiwari 1955). In Hooghly, the species occurs mainly in the middle and upper zones.

P. scabriculus has been recorded as occurring in various parts of South India (Henderson and Matthai, 1910); from "Malabar and the east coast of India upto the Mahanadi delta; the deltaic Bengals." (Tiwari 1955). In Hooghly, it is found mainly in the middle and upper zones.

P. mirabilis occurs in various localities of gangetic delta (Kemp 1917; Tiwari 1955). This species is distributed throughout the estuary though is landed in the catches mainly from the upper and middle zones.

MATERIAL AND METHODS

For studying the embryonic and larval development, live berried females obtained from the commercial catches were transported to the laboratory and reared in aquaria in fresh water. In a few instances where mature males and females were released in the aquaria, it was observed that extrusion of eggs in females had taken place subsequent to their introduction into the aquarium. To study the early embryonic development, microscopic examination of a sample of few eggs separated from the parent, at regular interval of 24 hours, was made. Thus, the development was traced upto hatching. Temperature readings of water in the aquaria were taken at regular intervals. Faxon (1879) working on *Palaeomonetes vulgaris*, observed that "eggs if detached from the mother die invariably, unless the enclosed embryo has nearly reached the point of hatching". A similar conclusion was arrived at by Nataraj (1947) working on *Palaeomon idae* Heller. However, in the course of the present studies, it has been possible to successfully rear to hatching, at least 50% of the eggs separated from the parent, in small glass bowls (capacity 350 c.c.) where changing of water and cleaning of eggs were done at periodic intervals.

The different stages assigned to the developing eggs in the descriptions given in the text, are based on the progressive changes in the colour of the egg-mass and the characteristics of the live embryo as observed with the aid of a research microscope.

The larvae of *P. rudis*, *P. mirabilis* and *P. scabriculus* survived for two to three days after the first moulting; in *P. malcomsonii* the larva did not moult to second stage. Various measurements of the larvae were taken from dorsal side after rendering them inactive by the addition of a few drops of 30% alcohol. (Fig. 11).

EMBRYONIC DEVELOPMENT

P. malcomsonii. Berried specimens of the species were available in the middle and upper zones of the river Hooghly in the months of May to September.

Stage 1: (1-4 days)

The fertilised eggs (Fig. 1) are found to be more or less elliptical in shape 12-14 hours after extrusion. They are deep yellow in colour and the individual eggs on an average measure 0.82 mm. \times 0.52 mm. The centre of the egg is darker than its periphery, and has a thin transparent membrane completely covering it. Distinct hexagonal markings are seen on the surface showing an advanced stage of cleavage.

The ventral plate becomes noticeable as a well demarcated cellular transparent region at one end of the egg approximately 48 hours after extrusion. By the next day this structure increases in size, and the rudiments of the embryonic region namely, a stout cephalic lobe in front and a narrow and elongated thoracico-abdominal lobe behind with prominences of appendage buds, are well differentiated.

On the 4th day (Fig. 2) the cephalic lobe has increased in size and is divided into a larger anterior optic rudiment, and a smaller posterior rudiment of the antennule. Two knob like prominences *viz.*, the antennal and mandible rudiments, are differentiated towards the posterior region. The thoracico-abdominal lobe gets further differentiated into a forwardly flexed, abdominal process or telson rudiment.

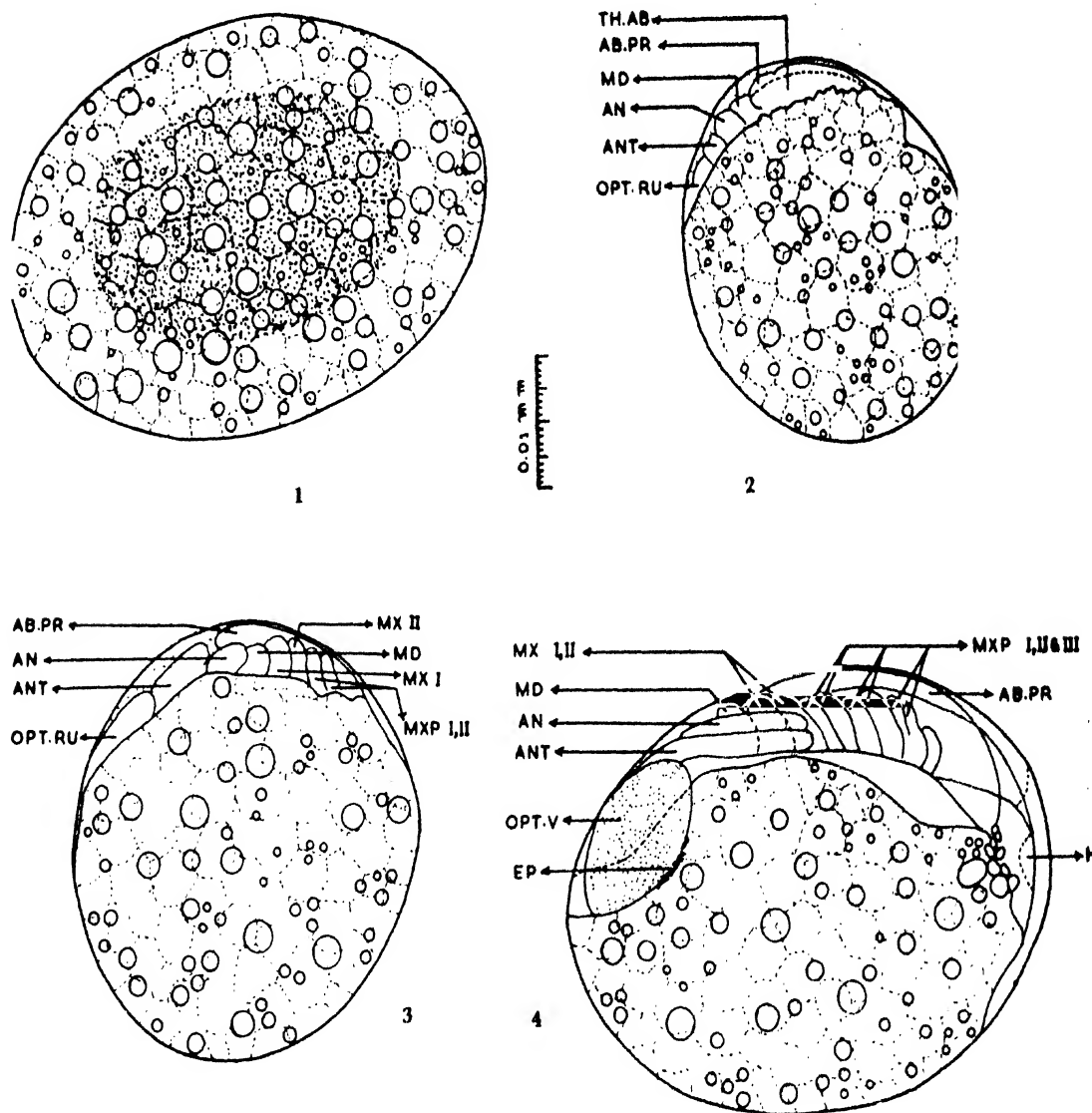


Fig. 1. Egg on the first day of development

Fig. 2. Egg on the 3rd day of development.

AB.PR: Abdominal process; AN: Antenna; ANT: Antennule; MD: Mandible;
TH.AB: Thoracico-abdominal lobe; OPT.RU: Optic rudiment.

Fig. 3. Egg on the 5th day of development

MX I and II: Maxilla, I and II; MXP: I, II: Maxillipeds I and II; rest as in Fig. 2.

Fig. 4. Egg on the 7th day of development

EP: Eye-pigment; H: Heart; MXP I, II and III: Maxillipeds I, II and III; OPT.V
Optic Vesicle. Rest as in Fig. 2.

Stage II : (5-6 days)

By the beginning of 5th day (Fig. 3) the rudiments of four more cephalic appendages, namely, the first and second maxillae and 1st and 2nd maxillipeds are also formed. Thus, a total of eight prominences are clearly visible on the embryonic region. The abdominal process is more pronounced and extends upto the antennal region, as a well-defined forwardly-directed process. This process is the only part of the embryo projecting away from the yolk, while the rest of the embryonic rudiments namely, the optic lobe and cephalic appendages, lie flat over the underlying yolk mass.

On the 6th day the optic rudiments are very conspicuous, lying flat over the yolk mass. The antennule and antenna are posteriorly directed. The mandible, the first and second maxillae, are still short and blunt, but directed downwards. The three maxillipeds are well defined by now. The abdominal process shows a cleft in its middle at posterior end. In the cephalo-thoracic region a tiny heart vesicle has made its appearance. At this region, a slightly elevated part of yolk mass of light yellow colour consisting of few large and small vacuoles is visible.

Stage III : (7-11 days)

From the 7th day to the time of hatching on the 14th day, the development appears mainly to consist of growth and further differentiation of the various regions and appendages already formed.

On the seventh day (Fig. 4) the heart vesicle begins to pulsate at irregular intervals in the beginning later on becoming more regular and rhythmic. The optic vesicle appears as a very thick and prominent structure and along its inner border, a short narrow and discontinuous dark streak of pigment becomes visible. The carapace has developed and covers the cephalo-thoracic region of the embryo. The appendages are elongated and tubular and the three maxillipeds appear distinctly bifid.

During the 8th and 9th days, the optic vesicles which are hitherto lying flat over the yolk begin to increase in size extending upwards on each side of the embryo. The eye-pigment has become continuous, oval shaped, and larger. The antennule and antenna have become more tubular, elongated backwards parallel to each other from the cephalic region to the region of the 1st maxilliped. The mandible and first and second maxillae remain short, but with slightly pointed ends, directed downward inclining slightly forwards. The three maxillipeds are more elongated. The yolk has diminished to more than half its original volume. Faint lines of segmentation are visible on the abdominal process which is seen at the level of maxillipeds; the gut is visible as a very narrow tube along its centre. The liberated fluid yolk which appeared on the 6th day consisting of number of vacuoles, has increased in volume and shows regular but slow movements.

By the 10th day (Fig. 5) the eye pigment becomes larger. The distal end of antennule is very finely pointed, and that of antennal exopodite is faintly annulated, and from the lower edge of the latter are seen very fine setae. The lateral edge of carapace is more distinct, curved upward posteriorly. Peristaltic movements of the liquid yolk are found to be more frequent and occasionally are seen movements of a few droplets of yolk inside the gut. Viewed from the lower surface of the egg, on the enlarged bifid posterior end of the telson are seen setae, as small protuberances.

Stage IV : (12th day to 14th day)

On the 12th day (Fig. 6) the embryo appears quite advanced. The yolk is highly reduced, appearing as 4 blocks, two anterior and two posterior. The embryo occupies the major portion of the egg. The eye pigment is almost spherical.

Telson has extended beyond the anterior end of the embryo curving upwards: viewed from the lower surface of egg, on its concave posterior end, the setae are well defined. The biramous maxillipeds have elongated, their distal ends becoming setose. The 1st and 2nd pereopods formed earlier, are visible as small tubular structures. The abdomen has six segments, the last one continuous with the telson. The embryo shows occasional jerky movements which became more frequent near the time of hatching.

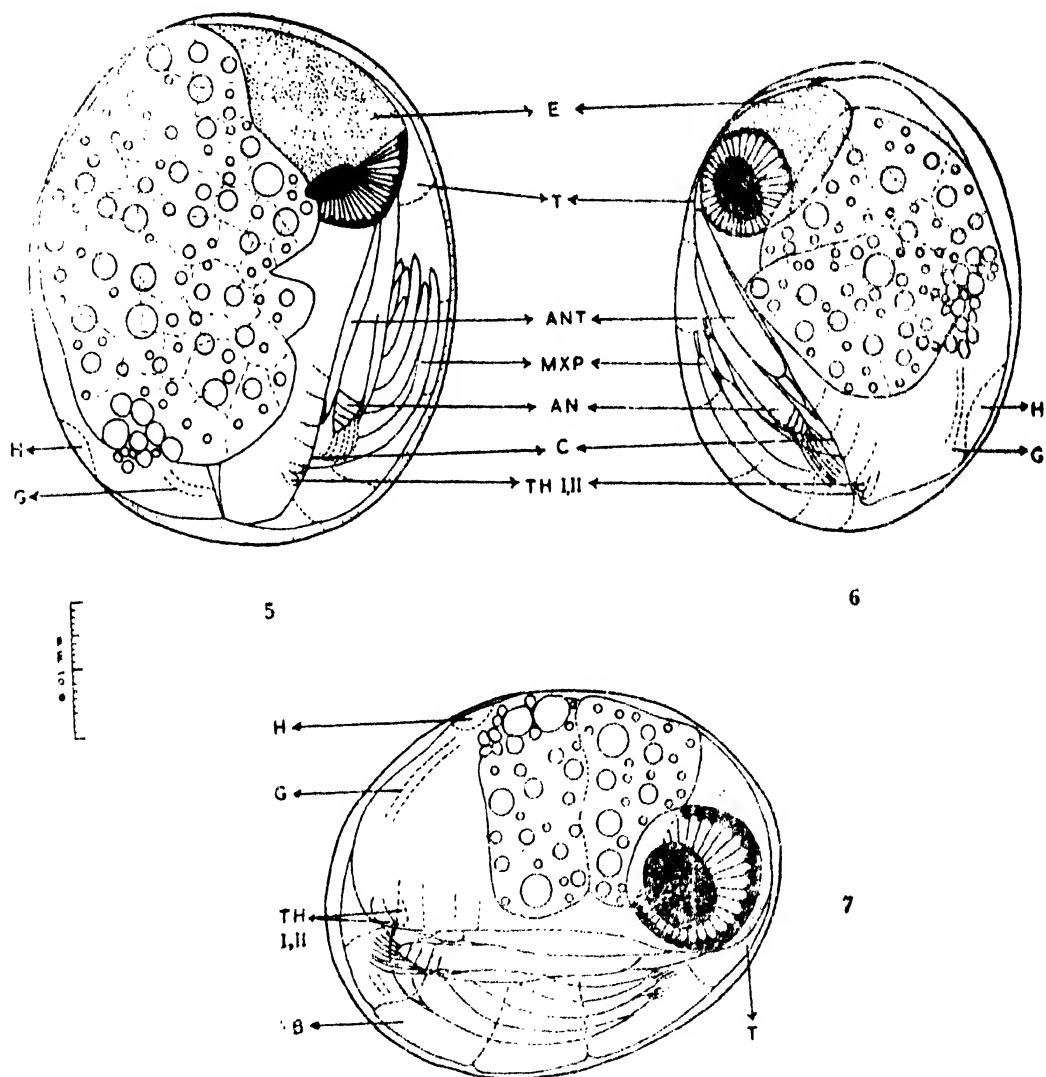


Fig. 5. Egg on the 10th day of development

C: Carapace; E: Eye; G: Gut; T: Telson; TH. I, II: Thoracic appendages (Pereopods) I and II. Rest as in Fig. 2 and 4.

Fig. 6. Egg on the 12th day of Development

Abbreviations as in Fig. 5.

Fig. 7. Egg on the 14th day of development.

AB: Abdomen. Rest as in Fig. 5.

The embryo continues to grow absorbing the remaining yolk, and by the 14th day (Fig. 7) the development is complete. The embryo is transparent, with a small amount of yolk beneath the carapace. Eye-pigment, completely spherical by now, is visible clearly to the naked eye. The heart-beat, peristaltic movements of the liquid yolk and the jerky motions of the embryo are more rapid.

Hatching

The larvae reared in aquaria hatched out late on the 14th day between 2.5 a.m. when the temperature was 25.5°C. At the time of hatching, the parent was found to swim constantly. The rapid movement of its pleopods along with the jerky movements of the embryo inside the egg membrane presumably help the process of hatching. The movements of the pleopods disperse the larvae. The larvae were hatched out in batches and after each batch is released, the parent rested for a while at the bottom of the aquarium slowing down its movements and after 2 or 3 minutes it swam up again to release and disperse a fresh batch of hatched larvae. The entire hatching took 2-3 hours in the aquarium.

By the next morning all the larvae were seen concentrated at one corner of the aquarium, some attached to the glass sides, all hanging head downwards.

Embryonic development in P. rudis, P. mirabilis and P. scabriculus

The general pattern of development in these three species bears a very close resemblance to that of *P. malcomsonii* which is described above. So, only the main points of differences noticed in the embryonic history of these species are indicated here.

P. rudis breeds in the middle and upper zones of the estuary from February to October with the peak period from June to October. The eggs take 19 days for development and hatching in temperature ranging from 25 to 32°C. The eggs are light green at the time of extrusion and measure 0.61-0.71 mm. (long axis) and 0.49-0.56 mm. (short axis). The ventral plate and embryonic rudiments appear on the third and fourth day respectively. The peristaltic movements of the liberated yolk are seen on the 9th day; and the pulsation of the heart vesicle on the 10th day, the streak of eye-pigment also appears on the same day.

P. mirabilis appears to breed all the year round in all the zones of the estuary. The eggs take 17-19 days in different broods to develop and hatch. The entire development and hatching took place when the water temperature in the aquarium ranged between 25 and 26°C in January 1958 and 28 and 29°C, in August 1958. The eggs which are deep orange in colour at the time of hatching, become lighter as the development advances, till finally they become almost transparent at the time of hatching. The measurements of the extruded eggs range from 0.54-0.61 mm. (long axis) and 0.41-0.46 mm. (short axis). The ventral plate and the embryonic structures make their appearance by the 4th and 5th day respectively. The streak of eye-pigment becomes visible on the inner border of the optic vesicle on the 9th day and the pulsation of the heart vesicle and the peristaltic movements of the liberated yolk become noticeable by the 10th day.

P. scabriculus appears to breed in the middle and upper zones from late July to January with a peak breeding period from August to November. The eggs take 20 days to develop and hatch into larvae at the temperature ranging from 22-23.5°C. They are brownish-yellow when extruded, becoming lighter as development advances and measure 0.46-0.59 mm. (long axis) and 0.37-0.41 mm. (short axis). The ventral plate and embryonic structures appear on the 3rd and 4th day of development, respectively. The pulsation of the heart and the peristaltic movements of the liberated yolk are noticeable on the 11th day. On the inner border of the optic vesicle, the streak of eye-pigment appears on the 10th day.

LARVAL DEVELOPMENT

Palaemon malcomsonii Stage I: (Fig. 8A and B). The larvae measure 2.06–2.28 mm. from the tip of the rostrum to the tip of telson. The rostrum is long and slender, bent towards the tip reaching more than half of the antennular peduncle. It measures 0.17–0.20 mm. from its base to the tip. Antero-lateral angles of the carapace are drawn out into small spines.

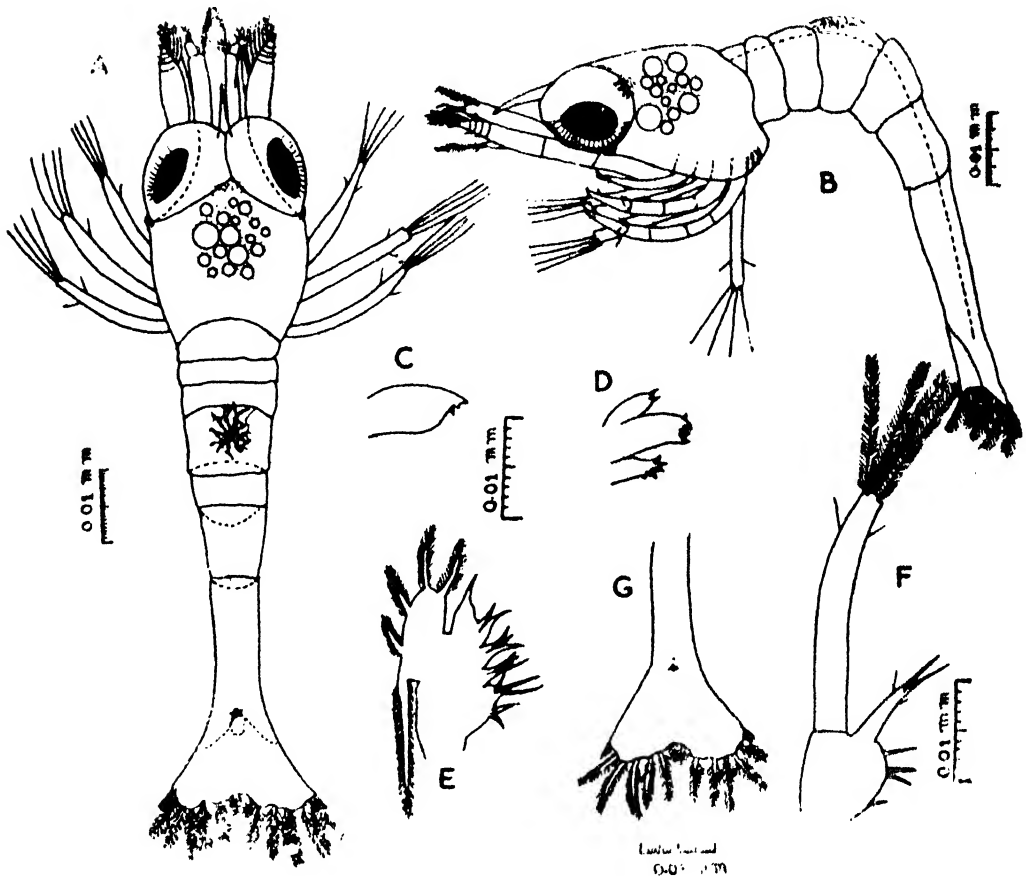


Fig. 8. A. 1st stage larva of *P. malcomsonii*: Dorsal view; B. Lateral view; C. Mandible; D. Maxilla I; E. Maxilla II; F. Maxilliped I; G. Telson.

Colouration: Under transmitted light, the larva appears transparent with light bluish pigment at the base of optic vesicles. A pigment spot of orange-red colour is seen at the base of each antenna and pink pigment on the oral region. There is a prominent orange-red dendritic chromatophore on the third abdominal segment, the branches of which extend on to the 2nd segment. A small chromatophore of the same colour at the base of telson. The eyes are large and sessile.

Antennule: The peduncle is long and unsegmented, with an outer unsegmented flagellum having 4 aesthetes and one short plumose seta. The inner flagellum is a long plumose seta.

Antenna : The peduncle has a small slender spine at the base. The flagellum starting from this region is elongated, unsegmented and about $2/3$ as long as the antennal exopodite, terminally bearing a long plumose seta and a small spine. There are 4 annulations terminally on the exopodite and 10 setae along its inner margin and apex the most distal one of which is small and spine-like. On the outer margin are two very small setae and there is a small papilla at the base of the 1st seta on inner margin.

Mandibles : (Fig. 8C) are small and short. The upper incisor part has 2 teeth and a slender and long tooth below them. The molar part is smooth, free of any teeth. The mandibles of both sides are similar in shape and size.

Maxilla I : (Fig. 8D). The endopod (palp) is small carrying two terminal spine-like setae; of the two lacinia (masticatory processes), the proximal one is narrow, less than half the size of the distal one. It carries 4 setae at its tip and one on its proximal margin, anteriorly. The distal process carries terminally 4 teeth, 2 of which are slightly larger, and a short seta.

Maxilla II : (Fig. 8E). The endopod has a terminal seta and a well-marked basal lobe carrying two setae. The protopodite with three masticatory processes each with three setae except the proximal large one, which carries 4 or 5 setae; the exopod (ealer) carries 5 plumose setae along its outer margin and apex, the most proximal one of which is the largest, and is directed backwards.

Maxillepod I : (Fig. 8F) has a slightly protuberant basis carrying 4 small setae on its outer margin. An unsegmented small and narrow endopodite carrying 3 terminal setae and a small one on its lateral margin. The exopodite has 4 plumose setae terminally and two setae, one on either side, a little lower down.

Maxillepod II : has a single seta on its slightly protuberant basis. The endopodite has four segments the last three of which are well demarcated. The third segment carries one seta on its margin and the last segment has 3 terminal setae and a basal one. Exopodite is as in the first maxillepod.

Maxillepod III : is similar to maxillepod II, except for the presence of 3 terminal and 2 basal setae on the last segment of endopodite. No setae are visible on the basis.

Percapoda I and II : are large folded biramous rudiments behind the 3rd maxillepod.

Telson : (Fig. 8G). is broad with concave posterior edge. It carries 7 spines on each lobe, the third and the fourth being the longest and the 7th the smallest.

The 1st stage larvae of P. rudis, P. scabrisculus and P. mirabilis

The I stage larvae in all the species, bear a very close resemblance to the corresponding stage described above and the stage described by Menon (1938) in *P. carcinus* and *P. rudis*. So, only the salient differences between I stage larvae of the various species including *P. rudis* (described by Menon 1938) are mentioned below :

P. rudis : The total length of the larva ranges from 1.92-2.06 mm. and that of the rostrum from 0.20-0.24 mm. The latter reaches to a little more than half of the antennular peduncle. The larva is transparent with light green yolk under the carapace. A tiny red spot at the base of the peduncle of antenna; blue and pink pigment at oral region; orange-red chromatophore on the mid-dorsal side of the third abdominal segment and pink and blue pigment around it.

P. scabrisculus : The total length of the larvae ranges from 1.76-2.01 mm. and that of the rostrum from 0.19-0.20 mm.; the latter reaches to little more than half of the antennular peduncle. The larva is transparent with light brownish-yellow yolk under the carapace. The larvae that hatched from different broods

in the aquaria, as well as those from tow-net collections do not show the presence of any chromatophores. The only colour visible is the red and blue pigment in the oral region.

P. mirabilis : The total length of the larvae ranges from 2.02 mm.-2.27 mm.; that of the rostrum from 0.20-0.22 mm. The rostrum reaches to a little more than half of antennular peduncle. The larva is transparent with light yellow yolk under the carapace. In transmitted light, the chromatophores and pigment are distributed as follows : At the base of each eye a thin streak of brown pigment and an orange-red chromatophore; orange-red pigment at the oral region ; a large finely-branched orange-brown chromatophore, one on each side of the third abdominal segment, the branches of which ramify all over the segment and extend on to the preceeding and succeeding segments ; pink and blue pigment in the mid-dorsal region of the same segment ; a very small orange-brown chromatophore on the telson, where it joins the 6th abdominal segment.

P. mirabilis : (Fig. 9A) Stage II larva

As mentioned earlier, the larvae survived to stage II in *P. rudis*, *P. scabriculus*, and *P. mirabilis*. Description of stage II larva of *P. mirabilis* is given below :

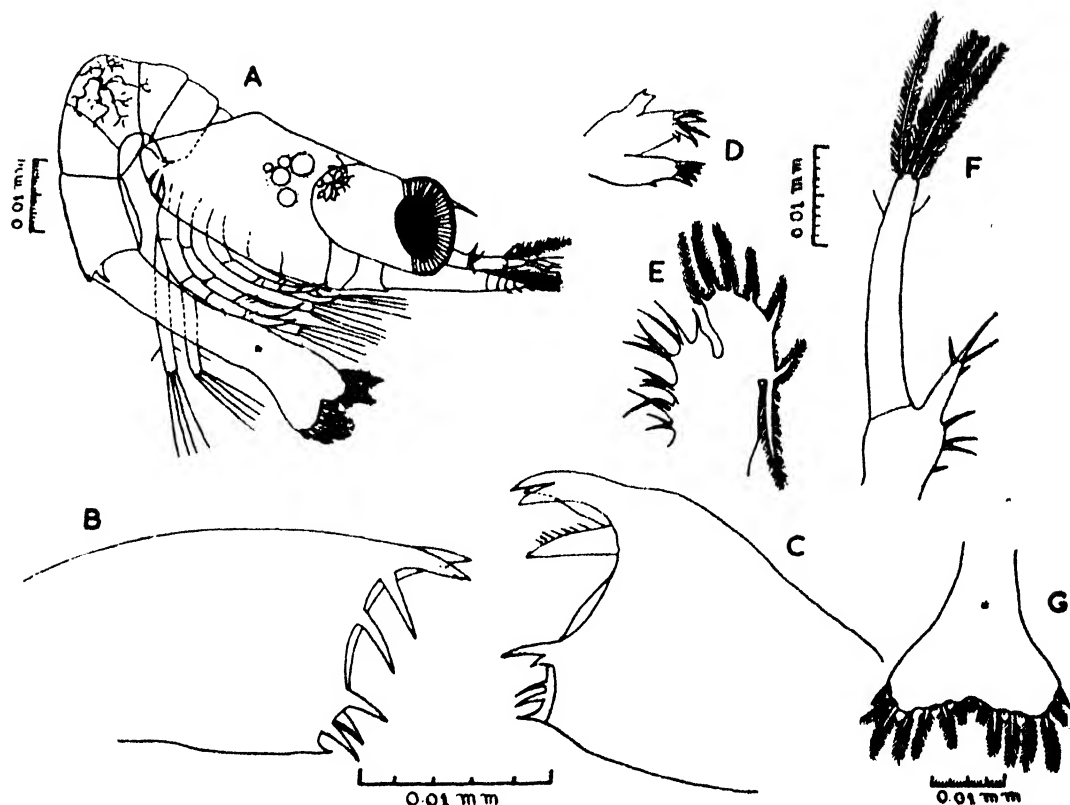


Fig. 9. A. 2nd stage larva of *P. mirabilis* : lateral view ; B. Mandible (right side) ; C. Mandible (left side) ; D. Maxilla I ; E. Maxilla II ; F. Maxilleped I ; G. Telson.

The 1st stage larva moulted at a temperature of 28°C. to the second stage, approximately 24 hours after hatching. The total length of the larva is 2.35 mm

and that of the rostrum 0.31 mm. Rostrum reaches nearly to half the length of antennular peduncle. A pterygostomial spine is present at each antero-lateral angle of the carapace, and a supraorbital spine on either side at the base of rostrum. A pair of dorso-lateral spines are seen on the 5th abdominal segment. An additional pair of tiny setae appear on the telson between the innermost pair of stage I. Pereiopods I and II are functional and folded biramous rudiments of pereiopods III and IV are seen behind them. Eyes are stalked and freely movable.

Colouration : In addition to the chromatophores present in the stage I, orange-red pigment appears at the basal segments of all the maxillepods as well as the first two pereiopods.

Antennule : The peduncle is segmented. The distal segment has two setae and the proximal one has a group of three setae terminally. Outer flagellum is unsegmented, bearing one plumose spine-like seta and 4 slender aesthetes. The inner flagellum is plumose and long.

Antenna : The scale (Exopodite) has 4 annulations distally and more than 10 setae on its inner margin and apex. Two small setae are present on its outer margin, and a papilla at the base of the first seta on its inner margin. The flagellum is long and slender with a spine opposite its base, and reaches to $2/3$ the length of the scale. It has a long terminal plumose seta and a small spine.

Mandibles : The right and left mandibles differ slightly. The incisor part of the right mandible (Fig. 9C) bears 3 short blunt teeth and the molar part 4-5 slender teeth. Between the two, a large spine serrated on its margin is present. The left mandible (Fig. 9B), has 2 large teeth on the incisor part and a long slender spine. The molar part bears 4-5 small teeth; between the two is a long and slender, nonserrated spine.

Maxilla I : (Fig. 9D). The distal masticatory process carries 4 large and 2 small spines and the proximal one 4-5 very fine spines and a minute one on its margin anteriorly. The endopod has two spine-like setae terminally.

Maxilla II : (Fig. 9E). The scale (exopod) bears 7 plumose setae, of which the proximal-most one is the largest. Protopodite carries three masticatory processes, the first of which bears 4 to 5 setae and the rest 3 setae each. The endopod has a terminal seta and a basal lobe carrying two setae.

Maxillepod I : (Fig. 9F). Basis bears three to four setae on its margin. The endopodite is unsegmented with three terminal setae and one lateral one. The exopodite has 6 setae, four terminally and two lower down one on each side.

Maxillepod II : With five-segmented endopodite having 2 setae distally on its third segment and one laterally on the last segment. Exopodite is as in the Maxillepod I.

Maxillepod III : With two setae on the basis. The endopodite is five-segmented; one seta is present on each of the first three segments, two setae on the fourth and 4 on the last segment terminally. Exopodite is as in the other maxillepods.

Pereiopods I and II : With five-segmented endopodites; one seta is present on each of the first three segments as above, and two on the fourth and last segments. Exopodites are as in the maxillepods.

Pereiopods III and IV : Are folded biramous appendages.

Telson : (Fig. 9G). Is broad and on its concave posterior border are present 8 setae on each lobe, the innermost one being the smallest.

The larvae survived for one day without moulting.

DISCUSSION

There are several points of similarity between the larvae of various species. However, some differences both in external features and morphometric characters do exist, by which it is possible to distinguish the larvae. Eight different morphometric characters as detailed in Table I and Fig. 11 are used in this study. *P. scabriculus* is different from all the other species in that it has no chromatophores on the third abdominal segment. *P. mirabilis* is characterized by the presence of one large dendritic chromatophore on each side, placed along the midlateral region of the third abdominal segment. The character that distinguishes both *P. malcomsonii* and *P. rudis* from the above two species is the presence of single large dendritic chromatophore placed middorsally on the third abdominal segment.

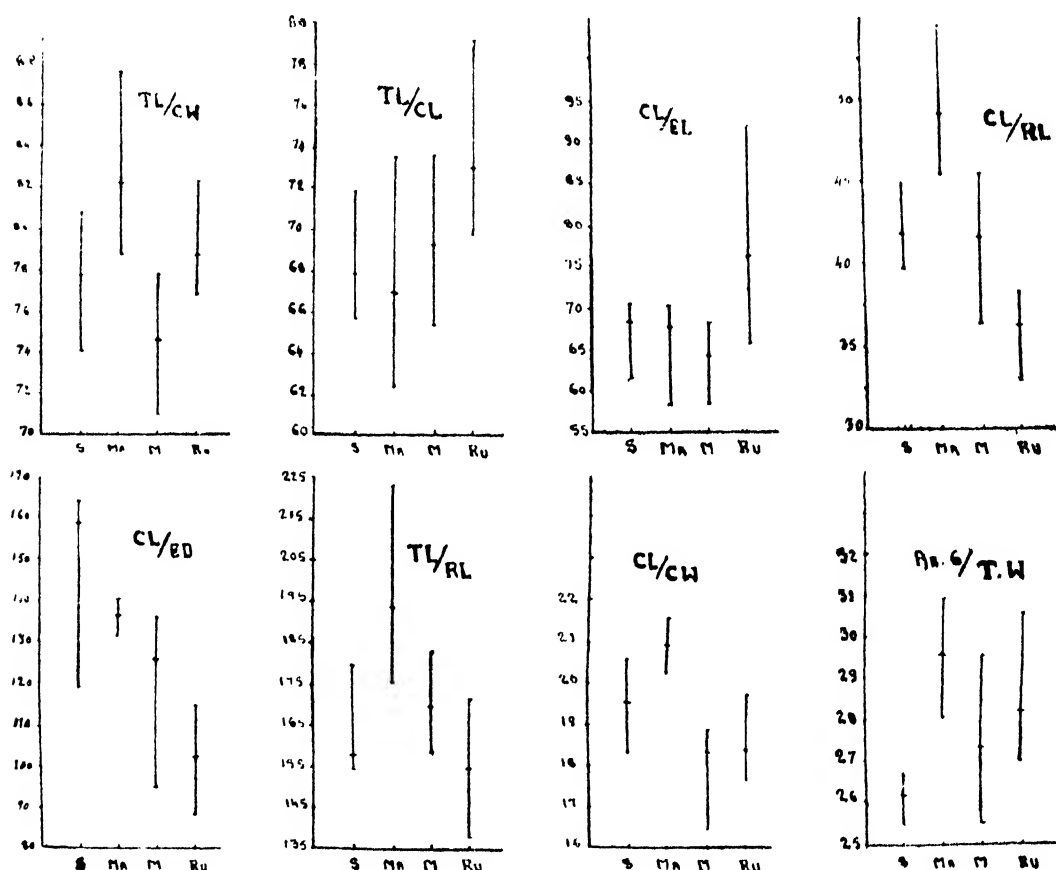


Fig. 10. Showing ranges and means of various morphometric characters—S: *P. scabriculus*; MA: *P. malcomsonii*; M: *P. mirabilis*; RU: *P. rudis*.

These last two species can be distinguished from each other only by the differences in certain morphometric characters, important among which are the ratios of Total length/Rostrum length, "Carapace" length/Rostrum length, "Carapace" length/Diameter of the eye pigment and "Carapace" length/width of cephalothorax at the region of eyes (vide Table I and Fig. 10). There are no significant differences

TABLE I
Showing the ranges and means of various morphometric characters

Total length/width of Cephalothorax at the region of eyes	Range Mean	74.12—80.55 μ 77.69 μ	78.71—87.55 μ 82.11 μ	70.89—77.69 μ 74.46 μ	76.84—82.28 μ 78.71 μ
Total length/"carapace" length	Range Mean	65.62—71.74 μ 67.66 μ	62.39—73.44 μ 66.98 μ	65.28—73.44 μ 69.19 μ	69.70—79.05 μ 72.93 μ
"Carapace" length/length of eyepigment	Range Mean	61.71—70.38 μ 68.34 μ	58.48—70.21 μ 67.83 μ	58.48—68.00 μ 64.33 μ	65.62—91.80 μ 76.16 μ
"Carapace" length/Rostrall length	Range Mean	39.61—44.88 μ 41.82 μ	45.39—54.40 μ 49.13 μ	36.55—45.39 μ 41.65 μ	32.81—38.25 μ 36.04 μ
"Carapace" length/width of eyepigment	Range Mean	119.00—164.39 μ 158.95 μ	131.75—140.25 μ 136.51 μ	95.20—136.00 μ 126.14 μ	88.40—114.75 μ 102.17 μ
Total length/Rostrall length	Range Mean	154.36—179.35 μ 157.76 μ	175.61—222.70 μ 193.12 μ	158.27—182.75 μ 169.66 μ	131.19—171.36 μ 154.46 μ
"Carapace" length/width of Cephalothorax at the region of eyes	Range Mean	18.36—20.57 μ 19.55 μ	20.23—21.59 μ 20.91 μ	16.49—18.87 μ 18.36 μ	17.68—19.72 μ 18.36 μ
Length of 6th abdominal Segment and telson/width of telson	Range Mean	25.50—26.69 μ 26.18 μ	28.05—30.94 μ 29.58 μ	25.50—29.58 μ 27.37 μ	27.03—30.60 μ 28.22 μ

in morphometric characters between *P. scabriculus* and *P. mirabilis*; and *P. mirabilis* and *P. rudis*. *P. mirabilis* differs from *P. malcomsonii* in respect of "Carapace" length/width of cephalothorax at the region of eyes, and Total length/width of

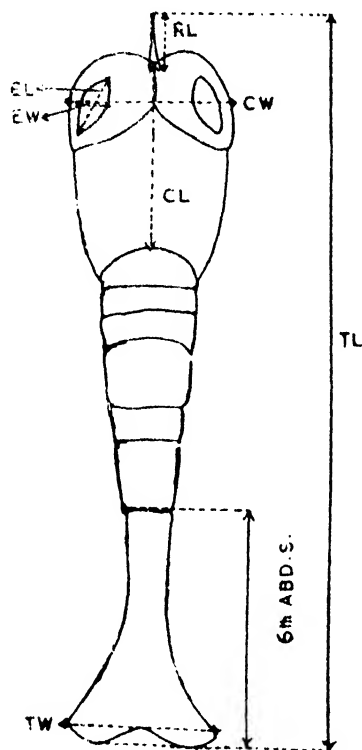


Fig. 11. Measurements used in this study.

cephalothorax at the region of eyes. *P. scabriculus* differs from *P. malcomsonii* in respect of "Carapace" length/Rostral length, and length of the 6th abdominal segment/width of telson; and from *P. rudis* in "Carapace" length/Diameter of eye-pigment, and the length of the 6th abdominal segment/width of telson, and "Carapace" length/Rostral length. (vide Table I and Fig. 10).

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EFFECT OF VARIOUS FACTORS ON THE INACTIVATION OF β -INDOLE ACETIC ACID *IN VITRO*

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ABSTRACTS

The experiments were designed to determine the effects of external factors on the inactivation of IAA *in vitro*. Solutions of chemically pure IAA were prepared and treatments carried out in flasks. Assay was done by root inhibition method using rice roots as test material.

The effects of the following factors alone or in combination with other were observed.

Autoclaving at 15 lbs. pressure for 20 mins. causes no marked variation in IAA content of the solution.

Addition of buffer to maintain a pH of 5.9 has a stabilizing effect on root growth.

Light at room temperature (27 \pm 2 C) has an inactivating effect on IAA even in the absence of sensitizing agent. Inactivation is maximum in white light followed by blue and green.

It increases with time and the rate is much higher in sunlight than in white light.

In darkness inactivation is related with time and temperature.

Organic acids added to IAA cause an acceleration of root growth.

INTRODUCTION

Two main theories have been put forward to explain tropic curvatures caused by unequal growth of an organ. While both schools support the idea that this is caused by a disbalance of auxin distribution; one of them considers that this is due to lateral transport of auxin while the other attribute it to the inactivation of auxin present. In recent years the lateral transport theory seems to have lost ground due to the work of Bunning *et al.* (1956). Their experiments with radioactive indole-acetic acid for testing the diversion of auxin stream by light give no support to the theory of auxin disbalance by transport. The authors explain phototropism as due to the activation or inactivation of the growth hormones and assume that the same applies for geotropism.

The inactivation of auxin *in vivo* and *in vitro* implies decrease in concentration which according to Gordon (1954) may be caused either by (a) the decomposition of the auxin molecule to yield free inactive products or by (b) adsorption or complex formation (binding) or both.

While pure auxin is inactivated by various agencies such as peroxides, hot dilute mineral acids, visible and invisible radiation etc., it is the latter agency to which is mainly attributed the role of auxin destruction in plants. It has been suggested by Gordon (1954) that appreciable photolysis of indole-acetic acid is possible only in the presence of intermediary pigments or sensitizers. A number of coloured compounds, of which the most important is riboflavin, has been shown to possess the function of a sensitizer, together with some fluorescent compounds, which absorb ultraviolet rays and fluoresce in the visible region (Ferri, 1951). This necessity of an intermediary pigment or sensitizer led to the formation of the "Riboflavin theory of photoreception" by Galston and Baker (1949). Accordingly riboflavin acts as a photoreceptor in plant cells in the photo-destruction of IAA, stimulating growth in darkness and inhibiting in light. This theory has received much adverse criticism in the last few years notably by Goldacre (1954), Gustafson (1948, 1953, 1954) and Mer (1954).

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In view of this contradiction it was thought necessary to probe into the still more fundamental facts of the direct and relative effects of light, temperature and time on IAA solutions *in vitro*. Although it has been repeatedly suggested by various authors that *in vitro* experiments do but little to elucidate the reaction occurring *in vivo*, but to strip down any reaction to its fundamentals *in vitro*, experiments are essential. The effect of light on pure IAA has been but rarely observed. Gordon (1954) reported slow photo-oxidation of IAA noticed by Algeus (1946) and himself; details are, however, not available.

In the present investigation an attempt has been made to correlate the various factors which are likely to bring about a disbalance in the auxin distribution. Amongst these the effect of light and temperature in relation to time and also of organic acids has been studied.

MATERIALS AND METHODS

For all the experiments performed β -indole-acetic acid (IAA) extra pure quality of E. Merck was used. The solid IAA when not in use was stored in a cool dark place to prevent decomposition. Assay of the quantity of IAA present in a given solution was made by the root inhibition test. As described previously (Sircar and Chakravarty, 1957) rice roots were used as the test material for the bioassay. The sprouted rice grains instead of being grown on agar slants were grown in liquid media of 20 c.c.t test solution in a test tube and by introducing a strip of filter paper into the solution, two grains were fixed to the moist filter paper 2.3 mm. above the liquid level. A set of ten test tubes containing 20 germinating seeds were prepared for each solution; main root lengths were measured after 48 hours' growth in complete darkness.

EXPERIMENTAL RESULTS

Expt. No. 1 : Effect of light, darkness, autoclaving, bacterial activity and pH on indole-acetic acid (IAA) :

A preliminary experiment was undertaken to observe the effect of continuous light at room temperature on IAA solution; other factors which might affect the results such as bacterial activity and pH were also taken into consideration.

In the experiments with light the intensity was not measured, but it comprised of one 40 watt fluorescent lamp at 4 ft. distance and one 100 watt tungsten lamp at 10 ft. distance from the flasks containing the solutions. This arrangement was used for all subsequent experiments in which light treatment was given. To check the effect of pH half of each solution was buffered before assay with Na_2HPO_4 ; KH_2PO_4 to maintain a pH of 5.9. Thus finally for assay for each concentration, the following sets were prepared, assay being carried out in sterilised test tubes.

- i. Distilled water as control.
- ii. Distilled water autoclaved at 15 lbs. pressure for 20 mins.
- iii. Distilled water buffered before assay.
- iv. Distilled water autoclaved and buffered.
- v. Fresh IAA solution of the concentration used for the treatment.
- vi. Fresh IAA of similar concentration autoclaved at 15 lbs. pressure for 20 mins.
- vii. Fresh IAA buffered before assay.
- viii. Fresh IAA autoclaved and buffered before assay.
- ix. IAA solution treated with continuous light for 120 hours.
- x. IAA autoclaved and then kept in light for 120 hours.

- xi. IAA kept in light for 120 hours and buffered before assay.
- xii. IAA autoclaved, then kept in light for 120 hours and buffered before assay.
- xiii. IAA kept in darkness for 120 hours.
- xiv. IAA autoclaved before keeping in darkness for 120 hours.
- xv. IAA kept in darkness for 120 hours, buffered before assay.
- xvi. IAA autoclaved, kept in darkness for 120 hours, buffered before assay.

From the results presented in Table I the following facts are evident :

- (1) Light at room temperature ($27 \pm 2^\circ\text{C}$) has an inactivating effect on IAA.
- (2) Even in darkness there is a certain amount of inactivation which is prominent even though not marked as in the case of light treated ones, hence other factors possibly time and temperature had a bearing on it.

TABLE I

Effect of Various Factors Light (120 hrs.), Darkness (120 hrs.), Autoclaving and Addition of Buffer—on IAA solutions (Concs. 0.01 mg./l, 0.1 mg./l and 1.0 mg./l) at $27 \pm 2^\circ\text{C}$

Set No.	Solution and treatment	Average root length in mm.	Percent- age of growth	Average root length in mm.	Percent- tage of growth	Average root length in mm.	Percent- tage of growth
I	Water	65.02	100.00	75.45 57.95*	100.00	55.02	100.00
II	Water autoclaved	63.82	98.21	67.45	89.30	55.72	100.04
III	Water buffered	59.15	91.00	52.65*	90.85	53.90	98.00
IV	Water autoclaved and buffered	83.40	126.00	55.09*	95.10	57.50	104.00
		IAA solution Conc. 0.01 mg/l		IAA solution Conc. 0.1 mg/l		IAA solution Conc. 1.0 mg/l	
V	IAA, fresh	45.75	70.40	24.35	32.10	27.30	49.62
VI	IAA, fresh, autoclaved	44.60	68.60	37.55	49.50	22.50	40.59
VII	IAA, fresh, buffered	47.80	73.51	35.68	47.30	30.79	55.92
VIII	IAA, fresh, autoclaved, buffered	47.95	73.72	33.90	58.50	23.75	43.26
IX	IAA 120 hrs. of light,	53.09	81.75	57.62	76.30	30.20	55.21
X	IAA autoclaved, 120 hrs. of light	61.89	95.26	65.45	85.42	50.89	92.00
XI	IAA 120 hrs. of light, buffered	55.65	85.62	57.60	76.23	29.65	53.00
XII	IAA autoclaved, 120 hrs. of light, buffered	60.25	92.50	50.39	87.02	47.80	86.82
XIII	IAA 120 hrs. of darkness	50.86	78.21	57.60	76.29	21.10	38.35
XIV	IAA autoclaved 120 hrs. of darkness	57.27	88.16	49.30	65.20	30.79	55.93
XV	IAA 120 hrs. of darkness, buffered	50.80	78.19	47.95	63.52	30.79	55.97
XVI	IAA autoclaved, 120 hrs. of darkness, buffered	59.26	91.12	47.80	82.50	35.53	43.25

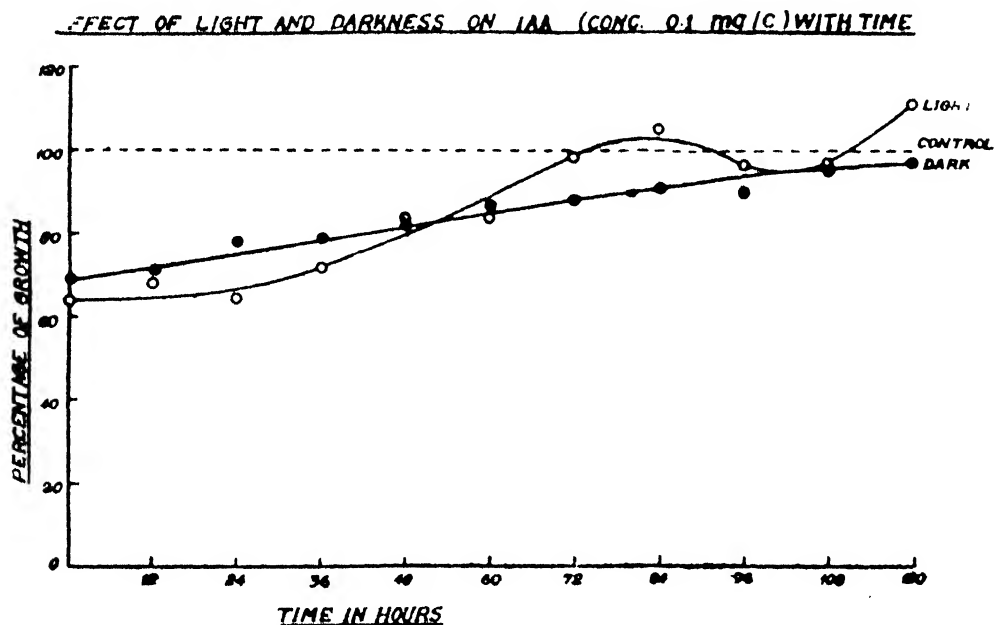
* These solutions had as their control an average root length of 57.95 mm. while the others had 75.45 mm. as length of control.

- (3) Autoclaving at 15 lbs. pressure for 20 mins. has no inactivation effect on 0.01 mg/L IAA as there is no significant difference observed in the case of the fresh solution which is autoclaved and which is not, but at other concentrations the results are inconsistent.
- (4) Addition of buffer has a stabilizing effect. Root growth was enhanced in certain cases due probably to the buffer acting as a nutrient.

Expt. No. 2 : Effect of light and darkness on IAA solution of concentration 0.1 mg/l at intervals of 12 hours

The effect of light and darkness was further followed up in relation to the time factor. 0.1 mg/l IAA solution was autoclaved. One part of the solution was kept in light and the other in darkness, at room temperature ($30 \pm 2^\circ \text{C}$). Assay was carried out every 12 hours for 120 hours. Buffer was added before assay, and a control of buffered distilled water was kept with each set.

The results (Fig. 1) of assaying IAA at 12-hour intervals, in both light and dark treated solutions, show that IAA becomes inactivated with time. It is also noticed that the rate of inactivation in light is significantly higher than in darkness. This leads to the conclusion that (a) light alone sensitizes the photoinactivation of auxin even in the absence of photoreceptors. It appears that after 72 hours the inactivation leads to a low value of IAA which promotes root growth; (b) since inactivation also takes place in darkness other factors such as time and temperature are also concerned.



TEXT-FIG. 1.

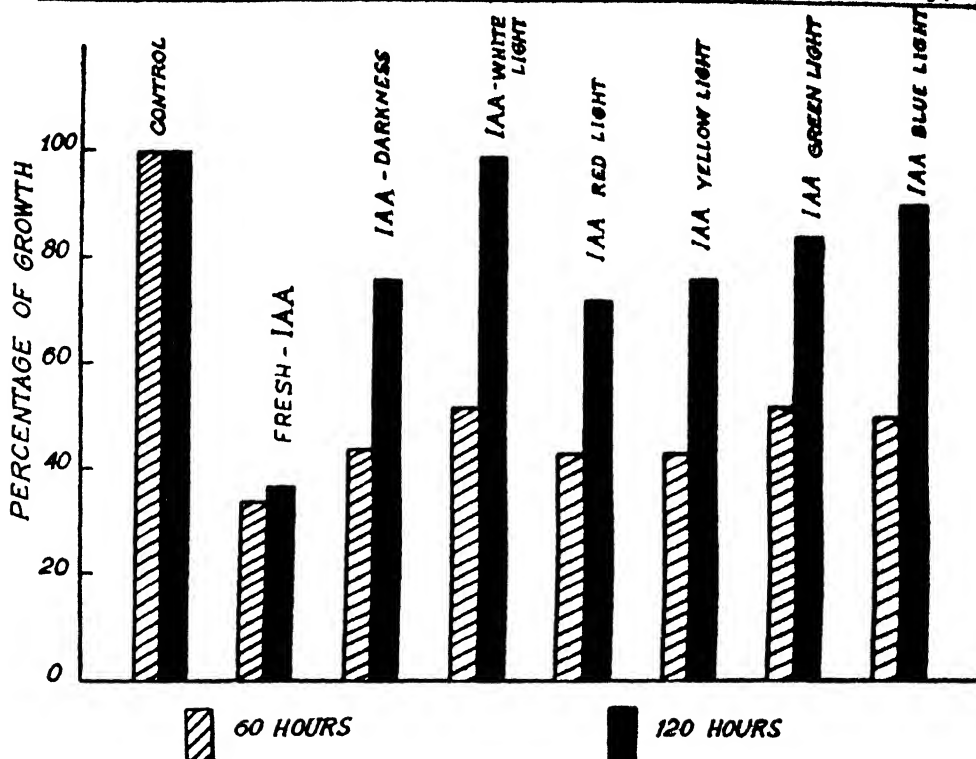
Expt. No. 3 : Effects of various coloured lights on IAA solution of 0.1 mg/l

The experiment was designed to determine which part of the visible spectrum was most concerned in the inactivating effect of light on IAA. Coloured glass filters for light were used but neither the exact values nor the intensities were determined due to unavailability of proper measuring instruments.

A solution of IAA of 0.1 mg/l concentration was prepared and autoclaved in flasks. Four of them were put into boxes with glass lids coloured red, yellow, green and blue respectively. The fifth solution was kept in a box with colourless glass lid for white light, and the sixth was wrapped in black paper and enclosed in a completely dark box with cardboard lid. They were kept at room temperature ($25 \pm 2^\circ\text{C}$) and assayed at 60-hour and 120-hour intervals, after buffering.

The results obtained are shown in Fig. 2. Maximum inactivation is noticed in white light being followed by blue and green light respectively. It was also noticed that inactivation in red and yellow light had a value near to that obtained, by keeping the solution in the dark.

EFFECT OF VARIOUS COLOURED LIGHTS ON IAA (CONC. 0.1 mg/c).



TEXT-FIG. 2.

Expt. No. 4: Effect of sunlight on IAA solution of concentration 0.1 mg/l

Previous experiments on photo-inactivation of IAA solution *in vitro* were performed in artificial light. In order to determine the effects of the natural light containing ultraviolet and infrared rays, an experiment was undertaken. The inactivation of 0.01 mg/l after the following treatment was assayed by the root inhibition method and the results are presented in Table II.

A (Set V)—Flasks containing IAA solution were kept in complete darkness at $32 \pm 2^\circ\text{C}$.

B (Set IV)—Flask kept in the sun but wrapped in black paper to prevent entry of light. Temperature variation was ($35 \pm 2^\circ\text{C}$) in day time and ($32 \pm 2^\circ\text{C}$) at night.

C (Set III) - Solutions exposed to direct sunlight for 8 hours a day from 8 a.m. to 4 p.m. Temperature variation as before.

Before setting up the experiment, fresh IAA solution (Set II) together with a control of distilled water (Set I) was assayed after buffering. At the end of the experiment the inactivation was noticed as before.

TABLE II
Effect of sunlight on IAA (Conc. 0.1 mg/l)

Time	Solution and treatment	Average root length in mm.	Standard error	Percentage of growth
0 hr.	Set I	Control - H ₂ O	57.96	± 6.73
	Set II	Fresh IAA	26.26	± 0.62
8 hrs.	Set I	Control - H ₂ O	57.20	± 3.00
	Set II	Fresh IAA	26.60	± 0.78
	Set III	IAA exposed to sun	41.13	± 1.86
	Set IV	IAA dark treatment at sunlight temperature at	22.00	± 2.82
	Set V	IAA dark treatment at room temperature	31.26	± 5.64
Sets III, IV and V kept in the dark at room temperature (32 ± 2°C) for 16 hrs.				
8 hrs.	Set I	Control - H ₂ O	56.66	± 0.05
	Set II	Fresh IAA	27.06	± 5.86
16 hrs.	Set III		46.46	± 1.55
	Set IV		33.06	± 9.00
	Set V		29.26	± 3.32
Sets III and IV kept in the sun (35 ± 2°C) for 8 hrs. Set V kept in the dark at room temperature (32 ± 2°C) for 8 hrs.				
8 hrs.	Set I	Control - H ₂ O	60.86	± 2.74
	Set II	Fresh IAA	21.60	± 1.19
16 hrs.	Set III		49.60	± 0.77
	Set IV		41.60	± 7.56
8 hrs.	Set V		42.33	± 2.15
Sets III, IV and V kept in the dark at 32 ± 2°C for 16 hrs.				
8 hrs.	Set I	Control - H ₂ O	58.66	± 1.47
16 hrs.	Set I	Fresh IAA	20.93	± 1.18
8 hrs.	Set III		54.46	± 2.21
16 hrs.	Set IV		45.20	± 2.18
	Set V		45.33	± 2.93
Sets III and IV kept in the sun (35 ± 2°C) for 8 hrs. Set V kept in the dark at 32 ± 2°C for 8 hrs.				
8 hrs.	Set I	Control - H ₂ O	63.66	± 4.16
16 hrs.	Set II	Fresh IAA	24.53	± 0.92
8 hrs.	Set III		60.46	± 2.47
16 hrs.	Set IV		45.13	± 1.27
8 hrs.	Set V		46.73	± 2.99

Sets III, IV and V were subsequently kept in complete darkness at room temperature (35 ± 2°C) for 16 hours after which they were assayed again together with a control (Set I) and a freshly prepared IAA solution. The sets III, and IV were then returned to the sun and V was kept in dark at room temperature for 8 hours.

The process was repeated for three consecutive days. The following observations are made from the data presented in Table II.

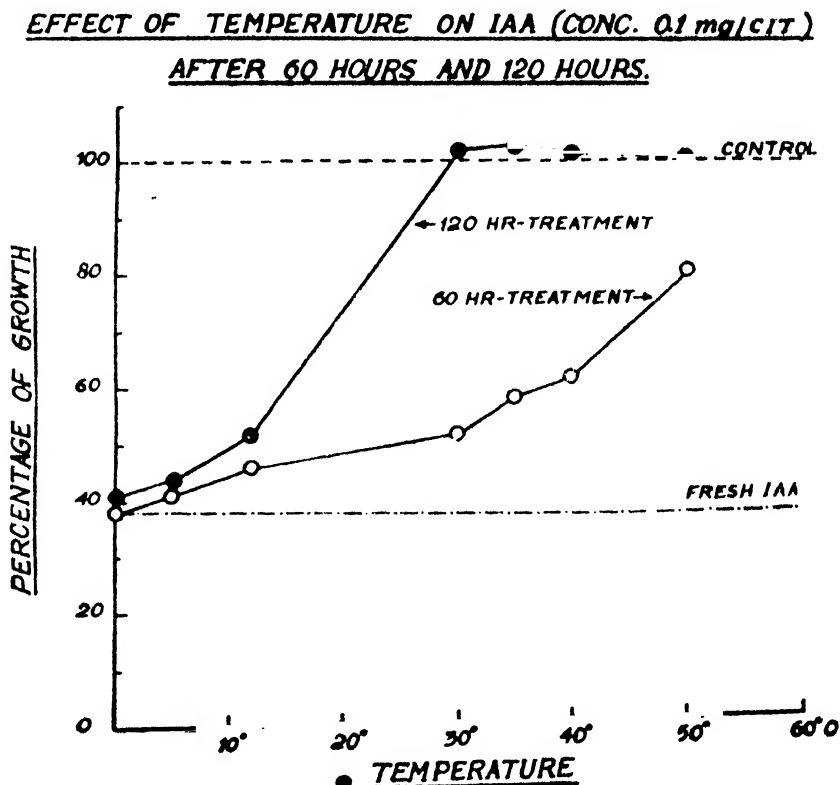
(1) In the direct sun, the rate of inactivation was maximum which might have been due to temperature as well as to the effect of the visible and the invisible rays.

(2) The solution that was kept in the sun after wrapping in black paper also showed a higher inactivation for first 24 hours than that placed in the dark at room temperature, but it was less inactivated in comparison to the one exposed to direct sunlight.

Expt. No. 5 : Effect of temperature on IAA in darkness.

IAA solution of 0.1 mg/l was kept in 500 c.c. flasks, which were plugged, wrapped with black paper and then stored at temperatures of 0°C, 5°C, 12°C, 30°C, 35°C, 40°C and 50°C. Assaying was done after 60 and 120 hours together with a fresh IAA of 0.1 mg/l and a control of distilled water. In each case the solution was buffered before assay. The results are presented in Fig. 3.

The following effects of temperature were noticed. (1) Inactivation of IAA is directly proportional to temperature; with an increase of temperature there is an increase in the amount of inactivation. (2) Time factor is also important as evident from the fact there is a slight inactivation at 0°C and 5°C.



TEXT-FIG. 3

TABLE III
Effects of Organic Acids on IAA

Conc. of IAA in mg/l	Conc. of acid in mg/l	CITRIC ACID			OXALIC ACID			SUCINIC ACID		
		Average root length in mm	Standard error	Percentage of growth	Average root length in mm	Standard error	Percentage of growth	Average root length in mm	Standard error	Percentage of growth
---	---	68.20	± 1.03	100.00	61.20	± 2.46	100.00	61.20	± 2.46	100.00
0.01	---	36.40	± 1.95	53.37	33.00	± 0.97	53.65	33.00	± 0.97	53.65
0.10	---	18.73	± 0.61	27.47	14.95	± 0.32	24.45	14.96	± 0.32	24.45
1.00	---	10.27	± 0.87	15.05	8.00	± 1.89	13.01	8.00	± 1.89	13.01
---	0.01	67.93	± 0.97	99.61	59.74	± 1.42	97.62	60.40	± 1.18	98.69
---	0.10	66.53	± 1.16	97.56	58.13	± 1.97	91.99	52.26	± 1.28	85.39
---	1.00	---	---	---	52.28	± 0.92	85.42	49.08	± 0.72	80.19
0.01	0.10	72.60	± 0.85	106.45	63.60	± 0.89	107.99	61.47	± 1.82	100.41
0.10	0.10	67.40	± 1.58	98.83	61.67	± 1.11	101.01	59.51	± 1.83	97.24
1.00	0.10	27.80	± 4.52	40.76	22.85	± 1.62	37.33	19.77	± 0.99	32.21
0.10	0.01	57.93	± 1.86	84.95	50.40	± 2.00	82.35	48.58	± 0.69	79.38
0.10	0.10	67.40	± 1.58	98.93	61.67	± 1.45	101.01	59.51	± 1.83	97.24
0.10	1.00	54.67	± 0.52	80.16	50.79	± 1.96	82.99	44.56	± 1.36	72.81

Expt. No. 6: The effect of organic acids (Citric, Oxalic, Succinic) on IAA.

This experiment was carried out in order to determine the direct effect of organic acids on IAA solutions. Solutions of oxalic acid, citric acid and succinic acid (pure quality of E. Merck) of concentrations 0.01 mg/l, 0.1 mg/l and 1.0 mg/l were prepared. The following sets with solutions of IAA (Conc. 0.01 mg/l, 0.1 mg/l, and 1.0 mg/l) were made. Assay was done after buffering to ensure that the effect of the acid was not due to its pH. Distilled water was used as control.

- i. Control--H₂O
- ii. IAA--0.01 mg/l
- iii. IAA--0.1 mg/l
- iv. IAA--1.0 mg/l
- v. Organic acid--0.01 mg/l
- vi. Organic acid--0.1 mg/l
- vii. Organic acid--1.0 mg/l
- viii. IAA (0.01 mg/l) + Organic acid (0.1 mg/l)
- ix. IAA (0.1 mg/l) + Organic acid (0.1 mg/l)
- x. IAA (1.0 mg/l) + Organic acid (0.1 mg/l)
- xi. Organic acid (0.1 mg/l) + IAA (0.1 mg/l)
- xii. Organic acid (0.1 mg/l) + IAA (0.1 mg/l)
- xiii. Organic acid (1.0 mg/l) + IAA (0.1 mg/l)

The results obtained are expressed in Table III.

- (1) The organic acids have a slight inhibitory effect on root growth.
- (2) The organic acids when present together with IAA in equal proportions cause neutralization of the effects of IAA, and sometimes show increase of growth over the control.
- (3) All the organic acids tend to show similar effect on IAA, causing increase of growth over that of IAA.

DISCUSSION

In the *in vitro* experiments different factors, which may have an inactivating effect on IAA, or affect auxin metabolism, were investigated. The factors may be grouped under three distinct heads:

- (a) Light in relation to time and temperature.
- (b) Temperature in relation to time.
- (c) Organic acids and their role in auxin metabolism.

Evidence has been presented here to indicate that IAA solutions have a tendency to become inactivated with time, at temperatures over 5°C. This inactivation, with passage of time, is noticed in both light and darkness, the rate being much higher in the former. The gradual inactivation in light was previously observed by two other workers. Gordon (1954), in his review on occurrence, formation and inactivation of auxins, mentioned that such an effect had been observed by him, though up to that time his data remained unpublished. He also referred that such a slow photo-oxidation of IAA was noticed by Algeus (1946). The slow inactivation of IAA in the dark too has not been reported elsewhere. Since IAA is a substance with no absorption band in the visible spectrum, appreciable photolysis can only be obtained in the presence of an intermediary pigment or sensitizer. Gordon suggests that the photoreceptor in this case is the product of oxidation itself. This may be the case, as it is seen in the experiments performed that the rate of inactivation is slow in the first 36 hours or so, afterwards the rate increases considerably; at the same time it is of interest to note that the rate is more equable in the case of the dark treatment. The question however remains as to the initial cause of

oxidation which forms products later concerned in the photolysis as the intermediary pigment, as also the fact that there is an appreciable inactivation even in the dark. A possible explanation of the data might lie in the fact that aqueous IAA solution has a tendency towards inactivation with time which, though slow, is yet appreciable and perhaps the agent which causes the primary inactivation leading to the formation of the photolytic intermediary. It is also suggested that the temperature may have a bearing on this phenomenon.

The effects of temperature, as demonstrated in the experiments carried out, show that with increase of temperature there is a decrease in the concentration of IAA in the dark which is also correlated with time. IAA is scarcely decreased in concentration at 0°C, even after 120 hours; on the other hand the solutions kept at 30°C and above were completely inactivated after 120 hours. The thermal inactivation of IAA in the dark has supporting evidence from the work of Mer (1951). His high temperature experiment shows a decrease in the total length of the treated *avena* seedling in relation to control; this effect might be cited as evidence for the destruction of auxin or any other growth promoting metabolite. However in this case there is an increase in the growth rate of the coleoptile and delayed response in the mesocotyl. Galston and Hand (1949) explained the reduction in the length of the pea epicotyl following treatment at 35°C and above, in terms of thermal destruction of adenine. In view of the wide distribution of adenine in plant cells Mer finds it difficult to explain the inverse growth of the different parts of the seedling on this basis.

Gregory and Hancock (1955) showed that both rate and amount of auxin transported are equally temperature dependent. The rate of transport of native auxin is almost nil at 0°C in shoots of apple and reaches a value of 1 cm./hr. near the optimum temperature of 27°C. In the range between 10°C and 30°C, the transport has a Q_{10} of the order of 2. In view of these facts, the increase of coleoptile length in the high temperature treatment, where there is general decrease in growth, may be explained on the ground that the concentration of the auxin in the coleoptile not only balances that of the thermal-inactivation but there is an increase in the native auxin resulting in accelerated growth. The increase in concentration may be due to the fact, that at certain temperature (up to 30°C), the rate of synthesis is high enough to balance not only increased inactivation but also increased rate and amount of transport. Above 30°C however both the rate and amount of transport are lowered and this is an additional factor which may influence increased IAA concentration in the coleoptile resulting in greater length. The mesocotyl on the other hand is not a seat of auxin synthesis. So here increase can only take place if the amount transported is greater than the amount inactivated. At temperature above 30°C however the transport amount as well as its rate decreases and simultaneously the rate of inactivation increases. These two effects together may account for a possible decrease in amount of auxin present and hence for the reduced mesocotyl growth.

In plants growing in the open, many physical factors may cause variation in its auxin content. Not only does the thermal effect come in but also sunlight containing the visible, ultraviolet and infra-red rays. These are potent auxin inactivator as evident from the fact that the treatment in the sun causes extremely rapid disappearance of IAA from the solution. From this it may be suggested that there is continuous inactivation of IAA *in vivo* caused by time, temperature and radiation, and in most cases this inactivation rate is balanced by the rate of synthesis. When it is not so, fluctuation in growth results.

Reinert (1952) pointed out particular difficulties of using root inhibition test for determining auxin activity of plant extracts and like materials because of the masking effect of acids, salts and other organic compounds present in the solution. It was thought that the free organic acids would have the most effect since it was

shown by Ulrich (1941), Bonner (1949), and Nitsch and Nitsch (1955) to be intimately linked with auxin metabolism through respiration.

Eberts, Burris and Riker (1951) working on the effects of IAA and common organic acids on the respiration of tomato stem and crown gall tissue slices showed that most of the acids tested increased respiration of untreated stem and gall slices both at 27°C and 32°C. In no case did the organic acid appreciably reverse the inhibition induced by 10^{-3} M IAA. Nitsch and Nitsch (1955) observed that the juice of tomatoes, which is known to contain a large proportion of organic acids, does not stimulate the growth of plant tissues unless auxin is added at the same time, but in case it is, the effect greatly exceeds that of auxin alone.

Though growth only has been noted in the experiments performed with organic acids and IAA on rice seeds, and respiratory rates has not been measured, yet the growth data are in accordance with the results obtained by other authors. Organic acids have an appreciable inhibitory effect at the concentration of 1 mg/L, but at lower concentrations the effect is almost nil. The inhibitory effect of IAA is still more marked over a wider range. It has also been noticed that organic acids when added to IAA cause a marked stimulation of growth, which in certain cases exceeds that of the control. The best neutralisation of the effects of IAA by organic acids is obtained when equal quantities of the two are mixed. In cases where the concentration of organic acid is lower, it is not able to neutralise the effect of the high IAA concentration, but in cases where the organic acid content is higher, stimulation is also observed but not so marked due probably to the fact that at high concentrations organic acids in themselves are inhibitory.

Karlsson and Eliasson (1955), on the basis of respiratory determinations on the elongating wheat root sections, suggested that in the absence of substrate, cell wall dissolution may actually occur in the extension region with the utilization of released organic products as respiratory substrate.

Torrey (1956) points out that root elongation is dependent on cellular metabolism including at least aerobic respiration and concomitant phosphorylation.

The conclusion, drawn from the previous work and the experiments performed here, is that organic acids stimulate auxin inhibited root growth by supplying respiratory substrate. One of the causes of auxin inhibition of root growth may be that not enough substrate is available for auxin induced respiration, which is known to be much higher than that of the normal rate, and respiration supplies the energy necessary for growth; retardation of this process would lower growth rate. When an organic acid is supplied it enters into the active metabolic cycle supplying additional substrate for increased auxin-induced respiration, thus promoting growth.

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